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SCIENTIFIC OPINION

Scientific Opinion on the risk posed by pathogens in food of non-animal origin. Part 2 (*Salmonella* and Norovirus in leafy greens eaten raw as salads)[1]

EFSA Panel on Biological Hazards (BIOHAZ)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Leafy greens eaten raw as salads are minimally processed and widely consumed foods. Risk factors for leafy greens contamination by *Salmonella* spp. and Norovirus were considered in the context of the whole food chain including agricultural production and processing. Available estimates of the prevalence of these pathogens (together with the use of *Escherichia coli* as an indicator organism) in leafy greens were evaluated. Specific mitigation options relating to contamination of leafy greens were considered and qualitatively assessed. It was concluded that each farm environment represents a unique combination of numerous characteristics that can influence occurrence and persistence of pathogens in leafy greens production. Appropriate implementation of food safety management systems, including Good Agricultural Practices (GAP), Good Hygiene Practices (GHP) and Good Manufacturing Practices (GMP), should be primary objectives of leafy green producers. The relevance of microbiological criteria applicable to production, processing and at retail/catering were considered. The current legal framework does not include microbiological criteria applicable at primary production which will validate and verify GAP and GHP. It is proposed to define a criterion at primary production of leafy greens which is designated as Hygiene Criterion, and *E. coli* was identified as suitable for this purpose. A Process Hygiene Criterion for *E. coli* in leafy green packaging plants or fresh cutting plants was considered and will also give an indication of the degree to which GAP, GHP, GMP or HACCP programs have been implemented. A Food Safety Criterion for *Salmonella* in leafy greens could be used as a tool to communicate to producers and processors that *Salmonella* should not be present in the product. Studies on the prevalence and infectivity of Norovirus are limited, and quantitative data on viral load are scarce making establishment of microbiological criteria for Norovirus on leafy greens difficult.

KEY WORDS

Escherichia coli, leafy greens eaten raw as salads, microbiological criteria, mitigation options, Norovirus, risk factors, *Salmonella*

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SUMMARY

The European Commission asked EFSA's Panel on Biological Hazards (BIOHAZ) to prepare a scientific Opinion on the public health risk posed by pathogens that may contaminate food of non-animal origin (FoNAO). The outcome of the first and second terms of reference, addressed in a previous opinion, were discussed between risk assessors and risk managers in order to decide which food/pathogen combinations should be given priority for the other three terms of reference. This is the first opinion out of five and addresses the risk from *Salmonella* and Norovirus in leafy greens eaten raw as salads. The addressed terms of reference are to: (i) identify the main risk factors for leafy greens, including agricultural production systems, origin and further processing; (ii) recommend possible specific mitigating options and to assess their effectiveness and efficiency to reduce the risk for humans posed by *Salmonella* and Norovirus in leafy greens and (iii) recommend, if considered relevant, microbiological criteria for *Salmonella* and Norovirus in leafy greens.

Leafy greens are defined as leaves, stems and shoots from various leafy plants which are eaten as vegetables, and for the purposes of this opinion, only those eaten raw will be considered. The major crop types of leafy greens are: 'lettuce' types, leafy brassicas, cabbage, Belgian endive and watercress. 'Lettuce'-type leafy greens can be harvested at different development states, e.g. as mature whole heads, as baby leaves or as multi-leaves. Leafy greens may be processed to obtain ready-to-eat products, and these steps include: selection, elimination of external leaves, cutting, cooling, washing, rinsing, dewatering, packaging and storage. Other types of processing (e.g. freezing, mashing and unpasteurized juicing, blending) are either never or very rarely used and are not further considered. Some of these products are subject to cooking, pickling and other processes but these are also outside the scope of this Opinion. Harvested leafy greens are not subjected to physical interventions that completely eliminate microbial contamination. Technologies currently available for use by the leafy greens industry fall short of being able to guarantee an absence of *Salmonella* or Norovirus on leafy greens at primary production

For the identification of the main risk factors for *Salmonella* and Norovirus in leafy greens, including agricultural production systems, origin and further processing, the BIOHAZ Panel concluded that the main risk factors for the contamination of leafy greens with *Salmonella* at primary production are diverse and include: (1) environmental factors, in particular proximity to animal rearing operations, seasonality and associated climatic conditions (e.g. heavy rainfall causing floods) that increase the transfer of pathogens from their reservoirs; (2) contact with animal reservoirs (domestic or wild life); (3) use of untreated or insufficiently treated manure or compost; (4) use of contaminated agricultural water (for irrigation or pesticide treatments); (5) cross-contamination by food handlers and equipment at harvest or on farm post-harvest. *Salmonella* tends to decline on the surface of leafy greens during primary production. Therefore contamination events close to harvest (e.g. by irrigation water, floods), at harvest (e.g. by food handlers) or on farm post-harvest (e.g. by cross-contamination via water or from equipment or by food handlers) are the most important risk factors at primary production. Internalization in leafy greens has been observed after artificial inoculation of high levels of *Salmonella* making it difficult to assess its importance under natural conditions.

The main risk factors for the contamination of leafy greens with Norovirus at primary production are diverse and include: (1) environmental factors, in particular climatic conditions (e.g. heavy rainfall or floods) that increase the transfer of Norovirus from sewage or sewage effluents to irrigation water sources or fields of leafy greens; (2) use of water for irrigation or pesticide treatment which has been contaminated by sewage; (3) contamination by food handlers or equipment at harvest or on farm post-harvest. Internalisation of Norovirus, or surrogate viruses, in plant tissues has been observed in experimental studies. However, the virus levels used in these experimental studies may be higher than those which could be encountered during crop production; furthermore, information on Norovirus internalisation gained through the use of surrogates should be interpreted with caution, as properties of different viruses may affect uptake into, or clearance from, plants.

For both *Salmonella* and Norovirus, processes at primary production which wet the edible portions of the crop represent the highest risk and these include spraying prior to harvest, direct application of fertilizers, pesticides and other agricultural chemicals and overhead irrigation. Subsurface or drip irrigation which results in no wetting of the edible portions of the plants are of lower risk.

During processing, water submersion of fresh-cut leafy greens in washing tanks presents a risk of cross-contamination. For *Salmonella*, this risk is reduced if disinfectants are properly used within the washing tank water. There are few studies with surrogate viruses, such as Murine Norovirus, that investigate the effectiveness of chemical inactivation of Norovirus in processing water. The effectiveness of chlorine against Norovirus is not fully defined due to the lack of an infectivity assay. During processing, contamination or cross-contamination via equipment, water or by food handlers are the main risk factors for contamination of leafy greens for both *Salmonella* and Norovirus. Adherence or biofilm formation of *Salmonella* on processing equipment may become a source of contamination for leafy greens and may be difficult to remove by routine cleaning methods. At distribution, retail, catering and in domestic or commercial environments, cross-contamination of items, in particular via direct or indirect contact between raw contaminated food of animal origin and leafy greens are the main risk factors for *Salmonella*. At distribution, retail, catering, in domestic and commercial environments, the Norovirus-infected food handler is the main risk factor. Although less documented than for Norovirus, contamination of leafy greens with *Salmonella* by food handlers is a potential risk. Norovirus can persist on leafy greens. Survival of *Salmonella* can occur on leafy greens and, under certain conditions of storage growth may occur especially on fresh-cut leafy greens.

For the recommendation of possible specific mitigating options and the assessment of their effectiveness and efficiency to reduce the risk for humans posed by *Salmonella* and Norovirus in leafy greens, the BIOHAZ Panel concluded that: appropriate implementation of food safety management systems including Good Agricultural Practices (GAP), Good Hygiene Practices (GHP) and Good Manufacturing Practices (GMP) should be the primary objective of operators producing leafy greens eaten raw as salads. These food safety management systems should be implemented along the farm to fork continuum and will be applicable to the control of a range of microbiological hazards. As *Salmonella* has reservoirs in domestic as well as wild animals, birds and humans, the main mitigation options for reducing the risk of contamination of leafy greens are to prevent direct contact with faeces as well as indirect contact through slurries, sewage, sewage sludge, and contaminated soil, water, equipment or food contact surfaces. Compliance with hygiene requirements, in particular hand hygiene, is an absolute necessity for food handlers at all stages of the leafy green production and supply chain to reduce the risks of both *Salmonella* and Norovirus contamination. Production areas should be evaluated for hazards that may compromise hygiene and food safety, particularly to identify potential sources of faecal contamination. If the evaluation concludes that contamination in a specific area is at levels that may compromise the safety of crops, in the event of heavy rainfall and flooding for example, intervention strategies should be applied to restrict growers from using this land for primary production until the hazards have been addressed. Each farm environment (including open field or greenhouse production) should be evaluated independently as it represents a unique combination of numerous characteristics that can influence occurrence and persistence of pathogens in or near fields of leafy greens. Among the potential interventions, both water treatment and efficient drainage systems that take up excess overflows are needed to prevent the additional dissemination of contaminated water. Since *E. coli* is an indicator microorganism for faecal contamination in irrigation water, growers should arrange for periodic testing to be carried out to inform preventive measures. All persons involved in the handling of leafy greens should receive hygiene training appropriate to their tasks and receive periodic assessment while performing their duties to ensure tasks are being completed with due regard to good hygiene and hygienic practices. Clear information (including labelling) should be provided to consumers on appropriate handling of leafy greens which includes specific directions for product storage, preparation, intended use, 'use-by' date or other shelf-life indicators.

For the recommendation, if considered relevant, of microbiological criteria for *Salmonella* and Norovirus in leafy greens throughout the production chain, the BIOHAZ Panel concluded that: the

current legal framework does not include microbiological criteria applicable at the primary production stage. It proposed to define criteria to validate and verify Good Agricultural Practices (GAP) and Good Hygiene Practices (GHP). These criteria were designated as Hygiene Criteria and are defined as criteria indicating the acceptable functioning at pre-harvest, harvest and on farm post-harvest production prior to processing. Hygiene Criteria should be considered as distinct from Process Hygiene Criteria, which are applicable to food business operators, although some or all of the minimal processing actions (cleaning, coring, peeling, chopping, slicing or dicing and washing) may be common to both primary producers as well as food business operators.

E. coli was identified as suitable for a Hygiene Criterion at primary production of leafy greens and could be considered for validation and verification of Good Agricultural Practices (GAP) and Good Hygiene Practices (GHP) and on the basis of this, growers should take appropriate corrective actions. A Process Hygiene Criterion for *E. coli* in leafy green packaging plants or fresh cutting plants will give an indication of the degree to which collectively GAP, GHP, GMP or HACCP programs have been implemented. A Food Safety Criterion for *Salmonella* in leafy greens intended to be eaten raw as salads could be used as a tool to communicate to producers and processors that *Salmonella* should not be present in the product. Testing of leafy greens for *Salmonella* could be limited to instances where other factors indicate breaches in GAP, GHP, GMP or HACCP programs. Noroviruses can be detected in leafy greens, but prevalence studies are limited, and quantitative data on viral load are scarce making establishment of microbiological criteria for these foods difficult. Information is lacking on the relationships between the occurrence of Norovirus as detected by real time RT-PCR, infectivity and the actual risk to public health.

The BIOHAZ Panel also recommended that: (1) there should be implementation and evaluation of procedures such as sanitary surveys, training, observational audits and other methods to verify hygiene practices for leafy greens; (2) further data should be collected to support *E. coli* criteria at both primary production and during processing of leafy greens. This should also include standardization of sampling procedures at primary production; (3) a more detailed categorisation of food of non-animal origin should be introduced to allow disaggregation of the currently reported data collected via EFSA's Zoonoses database on prevalence and enumeration of foodborne pathogens; (4) risk assessment studies are needed to define the level of hazard control that should be achieved at different stages of production systems. Such studies should be supported by targeted surveys on the occurrence of *Salmonella* and Norovirus at specific steps in the food chain; (5) ISO methods and technical specifications (including for alternative methods) for Norovirus detection in leafy greens should be further refined with regard to sampling, sample preparation, limit of detection and interpretation of results and (6) research should be undertaken with the aim of: a) developing infectivity assays for Norovirus and b) understanding the extent of *Salmonella* and Norovirus internalisation in plant tissue during crop production at natural exposure levels.

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BACKGROUND AS PROVIDED BY EUROPEAN COMMISSION

In May 2011 a major outbreak of Shiga toxin-producing *Escherichia coli* (STEC[4]) O104:H4 occurred in Germany. About 4,000 people were reported ill with symptoms and the outbreak resulted in the death of more than 56 people. Other countries reported a certain number of people becoming ill by the same strain, most of whom had recently visited the region of northern Germany where the outbreak occurred. At the end of June 2011, there was a second cluster in Bordeaux, France, which was caused by the same *Escherichia coli* strain. In both cases, investigations pointed to the direction of sprouted seeds.

According to the 2009 Zoonoses Report[5], the majority of verified outbreaks in the EU were associated with foodstuffs of animal origin. Fruit and vegetables were implicated in 43 (4.4 %) verified outbreaks. These outbreaks were primarily caused by frozen raspberries contaminated with Norovirus.

According to the US Centers for Disease Control and Prevention (CDC) 2008 report on surveillance for food borne disease outbreaks[6], the two main commodities associated with most of the outbreak-related illnesses originating from food of plant origin were fruits-nuts and vine-stalk vegetables. One of the main pathogen-commodity pair responsible for most of the outbreaks was Norovirus in leafy vegetables. The pathogen-commodity pairs responsible for most of the outbreak-related illnesses were *Salmonella* spp. in vine-stalk vegetables and *Salmonella* spp. in fruits-nuts. In addition, as recently as September 2011, a multistate outbreak of listeriosis linked to cantaloupe melons caused 29 deaths in the US.

Regulation (EC) No 852/2004 on the hygiene of foodstuffs[7] lays down general hygiene requirements to be respected by food businesses at all stages of the food chain. All food business operators have to comply with requirements for good hygiene practice in accordance with this Regulation, thus preventing the contamination of food of animal and of plant origin. Establishments other than primary producers and associated activities must implement procedures based on the Hazard Analysis and Critical Control Points (HACCP) principles to monitor effectively the risks.

In addition to the general hygiene rules, several microbiological criteria have been laid down in Regulation (EC) No 2073/2005[8] for food of non-animal origin.

Following the STEC O104:H4 outbreak in Germany and France, the Commission already has asked EFSA for a rapid opinion on seeds and sprouted seeds. EFSA adopted a scientific opinion on the risk posed by STEC and other pathogenic bacteria in seeds and sprouted seeds on 20 October 2011. The current mandate intends to supplement the adopted opinion.

In view of the above, there is a need to evaluate the need for specific control measures for certain food of non-animal origin, supplementing the general hygiene rules.

TERMS OF REFERENCE AS PROVIDED BY EUROPEAN COMMISSION

EFSA is asked to issue scientific opinions on the public health risk posed by pathogens that may contaminate food of non-animal origin such as fruit, vegetables, juices, seeds, nuts, cereals, mushrooms, algae, herbs and spices and, in particular:

1. To compare the incidence of foodborne human cases linked to food of non-animal origin and foodborne cases linked to food of animal origin. This ToR should provide an indication of the proportionality between these two groups as regard human cases and, if possible, human burden.

[4] Also known as Verocytotoxin-producing *Escherichia coli* (VTEC).
[5] EFSA Journal 2011;9(3):2090
[6] www.cdc.gov/mmwr/preview/mmwrhtml/mm6035a3.htm?s_cid=mm6035a3_w
[7] OJ L 139, 30.4.2004, p. 1
[8] OJ L 338, 22.12.2005, p. 1

2. To identify and rank specific food/pathogen combinations most often linked to foodborne human cases originating from food of non-animal origin in the EU.

3. To identify the main risk factors for the specific food/pathogen combinations identified under ToR 2, including agricultural production systems, origin and further processing.

4. To recommend possible specific mitigating options and to assess their effectiveness and efficiency to reduce the risk for humans posed by food/pathogen combinations identified under ToR 2.

5. To recommend, if considered relevant, microbiological criteria for the identified specific food/pathogen combinations throughout the production chain.

The Commission would like an opinion on the first and second terms of reference by the end of December 2012. The outcome of the first and second terms of reference should be discussed between risk assessors and risk managers in order to decide which food/pathogen combinations should be given priority for the other terms of reference.

CLARIFICATIONS OF THE TERMS OF REFERENCE 3 TO 5 OF THE REQUEST ON THE RISK POSED BY PATHOGENS IN FOOD OF NON-ANIMAL ORIGIN

BACKGROUND AS PROVIDED BY EUROPEAN COMMISSION

On 23 January 2012, a request was provided to the European Food Safety Authority (EFSA) to issue scientific opinions on the public health risk posed by pathogens that may contaminate food of non-animal origin (FNAO).

The BIOHAZ Panel of EFSA adopted during its meeting on 6 December 2012 an opinion on the first and second terms of reference, focussing on

- the comparison of the incidence of food-borne human cases linked to FoNAO and food-borne cases linked to food of animal origin;

- identifying and ranking specific food/pathogen combinations most often linked to food-borne human cases originating from FoNAO in the EU.

It was agreed in the original request that the outcome of the first and second terms of reference should be discussed between risk assessors and risk managers in order to decide which food/pathogen combinations should be given priority for the other terns of reference addressing risk factors, mitigation options and possible microbiological criteria.

The first opinion of EFSA under this request identifies more than 20 food/pathogen combinations in its five top ranking groups. The opinion also contains a preliminary assessment of risk factors linked to certain examples of FoNAO (e.g. tomatoes, watermelons and lettuce), representing specific production methods for several FoNAO. Several risk factors and mitigation options may be common for several food/pathogen combinations due to similar production methods. It seems therefore opportune to combine the risk assessment of such food/pathogen combinations. When risk factors and mitigation options are identified as more specific to the individual food/pathogen combination, then these should be considered to supplement this approach and added where possible within the, opinions. Alternatively, it is worth mentioning that a reference could be made if such specific risks have already been addressed in previous opinions.

TERMS OF REFERENCE AS PROVIDED BY EUROPEAN COMMISSION

EFSA is asked, in accordance with article 29 of Regulation (EC) No 178/2002[9], to provide scientific opinions on the public health risk posed by pathogens on food of non-animal origin as regards risk factors, mitigation options and possible microbiological criteria. When considered more appropriate e.g. because of low prevalence of the pathogen or in view of a broader process control, indicators may be proposed as Process Hygiene Criteria. When addressing mitigation options at primary production, attention should be paid to Article 5(3) of Regulation (EC) No 852/2004[10], which laid down that the application of hazard analysis and critical control points (HACCP) principles shall only be applied to food business operators after primary production and associated activities[11]. This provision does, however, not exclude proposing microbiological criteria in accordance with terms of reference 5 when considered relevant.

EFSA is requested to provide opinions in line with the agreed terms of Reference 3 to 5 (EFSA-Q-2012-00237) for the following food/pathogen combinations with a similar production system:

(1) The risk from *Salmonella* and Norovirus in leafy greens eaten raw as salads.
Cutting and mixing before placing on the market should be included as potential risk factor and specific mitigation options proposed if relevant.

(2) The risk from *Salmonella*, *Yersinia*, *Shigella* and Norovirus in bulb and stem vegetables, and carrots.

(3) The risk from *Salmonella* and Norovirus in tomatoes.

(4) The risk from *Salmonella* in melons.

(5) The risk from *Salmonella* and Norovirus in berries.

[9] OJ L 31, 1.2.2002, p.1
[10] Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs.
[11] See guidance at: http://ec.europa.eu/food/food/biosafety/hygienelegislation/guidance_doc_852-2004_en.pdf

ASSESSMENT

1. Introduction

Leafy greens eaten raw as salads represent a minimally processed, ready-to-eat food commodity which is widely consumed and generally free from noxious substances such as poisonous chemicals, toxins and pathogenic microorganisms. However, the previous EFSA Opinion (EFSA Panel on Biological Hazards (BIOHAZ), 2013), ranked the risk of the combination of this food product together with *Salmonella* spp. and Norovirus, as of highest importance for human cases of infection originating from food of non-animal origin in the EU. The main risk factors, together with their mitigation options are applicable to many points in the food chain for leafy greens. However, since leafy greens eaten raw as salads do not include any processing steps or control points which will ensure removal or inactivation of biological hazards, it is particularly important to consider risk factors (and consequentially mitigation options) at the point of production. This property is also common to other foods of non-animal origin which are minimally processed and ready-to-eat, as well as some foods of animal origin (e.g. unpasteurised dairy products, shellfish and meats which are eaten raw). The approaches used in this opinion are:

1. To provide a descriptive analysis of the whole production process for a representative range of leafy greens which considers their origins in agricultural production, growing, harvesting, processing, distribution, retail, catering and handling in domestic environments. Risk factors for contamination by *Salmonella* spp. and Norovirus will be considered in the context of the agricultural production, processing, distribution and retail/catering/domestic environments. On a request from the European Commission, a brief comparison identifying possible differences in production systems and practices between the US and EU is included. Furthermore, following discussions with the European Commission it was agreed that for all the FoNAO considered in these related opinions, only minimally processed products will be considered (which includes those subject to cutting, washing, peeling, shredding, freezing, mashing and unpasteurized juicing or blending). Products undergoing thermal treatments (including blanching) as well as shelf stable juices are not considered in the scope of these opinions.

2. To assess specific mitigation options, separate sections are included relating to *Salmonella* spp. or Norovirus contamination of leafy greens eaten raw as salads. The assessments of the mitigation options were performed in a qualitative manner similar to that performed for the Scientific Opinion on the risk posed by Shiga toxin-producing *Escherichia coli* (STEC) and other pathogenic bacteria in seeds and sprouted seeds (EFSA Panel on Biological Hazards (BIOHAZ), 2011b).

3. Sampling and analytical methods for the detection of *Salmonella* spp. and Norovirus (together with the use of *Escherichia coli* as an indicator organism) in leafy greens eaten raw as salads were considered together with, where available, estimates of their respective prevalence. The relevance of microbiological criteria applicable to production, processing and at retail/catering were considered.

2. Production of leafy greens eaten raw as salads

2.1. Definition of leafy greens

Leafy greens are defined as leaves, stems and shoots from various leafy plants which are eaten as vegetables, and for the purposes of this opinion, only those eaten raw will be considered. This type of produce includes leafy green or any combination thereof that has been physically altered but remains in the fresh state and eaten with little or no subsequent treatment. Leafy greens were defined in a previous opinion (EFSA Panel on Biological Hazards (BIOHAZ), 2013) and include: beet greens, bitterleaf, bok choy, cabbage, celery, celtuce, Ceylon spinach, chard, chicory, Chinese cabbage, collard greens, cress, endive, epazote, garden cress, garden rocket, komatsuna, lamb's lettuce, land

cress, lettuce, mizuna greens, mustard, New Zealand spinach, pak choy, radicchio, rapini, spinach, tatsoi, watercress, water spinach and wrapped heart mustard cabbage among others. There are thus a great variety of leafy greens which can be classified according to their botanical names (Linnaeus species designation) but are in trade and by consumers usually best known by their common (Arabic) names. But the common name or designation of a leafy green vegetable may also differ or be understood differently depending upon the region or country. For example (*Cichorium intybus* L.) is well known as *witloof* (in Dutch) or *witlof* ('white leaf') but indicated as *indivia* in Italy, *chicory* in the UK, and as *endive* or *chicon* in France. Upon classification of leafy greens by their botanical names (Linnaeus species designation) it is however to be noted that within a botanical species also a further range of varieties, subvarieties and formae exist, some of them being more popular than others in some regions and changes in their production of varieties often differs from one production year to another. As such, leafy greens encompass a wide and continuously changing assortment of species and varieties.

Recently, the EFSA Panel on Biological Hazards (BIOHAZ), 2013 identified these products amongst the five top ranking groups of food/pathogen combinations according to specific criteria in the EU, and leafy greens eaten raw as salads were considered the highest priority in terms of fresh produce safety from an EU perspective.

Among this large variety of leafy greens one can distinguish between various leafy green types including:

- 'lettuce' types (*Lactuca sativa* L.- iceberg and romaine lettuce; *Cichorium endivia* L. - endive*; Beta vulgaris* L. - chard; *Valerianella locusta* (L.) Betcke - lambs lettuce; *Cichorium intybus* L.- red chicory*; Eruca vesicaria* subsp. *sativa* (Mill.) Thell. - rucola and *Spinacia oleracea* L. - spinach);
- leafy brassicas (*Brassica rapa* L. - Chinese cabbage, and *Brassica oleracea* L.- kale);
- cabbage (*Brassica oleracea* L. - green red and savoy cabbage);
- Belgian endive (*Cichorium intybus* L.) and
- watercress (*Nasturtium officinale* L.) (Appendix A, Freshfel, 2013).

'Lettuce'-type leafy greens can be harvested at different physiological states, e.g. as mature whole heads, as baby leaves or as multi-leaves (Figure 1). Mature whole heads, such as mature iceberg lettuce, are harvested when heads have developed the appropriate density and market size. For instance, processing specifications for mature iceberg lettuces require a core length around 7.5 cm. Harvesting is conducted before heads bolt, crack, yellow, or turn bitter. Baby-sized leaves are young leaves and petioles of any leafy green also known as small tender shoots, which are collected with a length from 10 to 15 cm. Therefore, baby leaves are leafy greens that are harvested at an immature stage and for this reason, production cycles are usually very rapid taking 35-60 days from sowing. In this case, seed mixes of different varieties are planted, and the varieties tend to vary during the season and according to consumer demand. Most of the growers buy specific seed lots and prepare the seed mixes themselves. Baby leaves are planted and grown similarly to standard varieties of whole heads with the exception of the plant density and the size. Baby leaves are physically smaller than whole heads. For baby leaves, sowing is usually performed directly on beds using a very high plant density of 800 plants m^{-2} (Selma et al., 2012). Multi-leaf is a relatively new salad concept. In this case, lettuce crops are planted with a plant density of 30 plants m^{-2}, which is higher than for whole heads (7 plants m^{-2}) but lower than baby leaves (Figure 1). Both leafy greens, baby and multi leaves, are characterized by a greater number of smaller plants than conventionally grown lettuce (Figure 1). The main advantages of baby and multi leaves are that a very large number of leaves of similar dimensions can be cut from one lettuce plant. Baby and multi-leaves, with many small leaves sprouting from a common stem are removed with a single cut with further cutting not being required. Additionally,

since the stem diameter is smaller than with whole head types, a lower wound response can be expected, with less bruising and minimal oxidation. Both Baby and Multi leaf usually have a similar leaf size of about 10 cm on average, however, baby leaves will be harvested at an earlier, immature stage during the crop cycle (Figure 2). Some authors have reported that although baby and multi leaf lettuces are subjected to far less wound damage than the shredded lettuce from the whole-head, the leaf age plays an important role in increasing respiration rate and determining postharvest quality, suggesting multi-leaf as a better material for ready-to-eat leafy greens (Selma et al., 2012).

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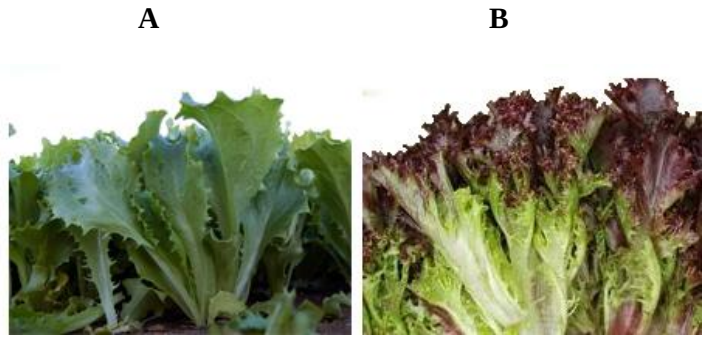
Figure 1: Open field cultivation of whole head lettuce (romaine lettuce) (A) baby lettuce leaf (lollo biondo) (B) and multi-leaf lettuces (read oak leaf) (C).



Source: Ana Allende, reprinted with permission of Quality and Safety Lab CEBAS-CSIC

Figure 2: Harvested baby (A) and multi-leaf (B) lettuces.

2.1.1. Seed and seedling production

The seed production involves pre-harvest and on farm post-harvest activities such as field preparation, planting, growth (including flowering and seed setting), irrigation, fertilization, pollination, swathing, field drying, seed harvest, storage and transport. Seed producers are involved in all parts of the leafy green production chain (FSANZ, 2010). Plants for seed production are grown in typical agricultural environments and seeds are generally treated as raw agricultural products. A wide range of seeds are used for leafy greens and thus a diverse range of agricultural practices may be associated with their production. Some growers may modify some of these practices depending on many factors, such as the needs of the crop, resources of the operation, and requirements, if any, imposed by the buyer or distributor (FSANZ, 2010; NACMCF, 1999).

To minimize damage to seeds during harvest, the plant material may be allowed to dry for a number of days until the moisture content falls to the desired percentage (i.e. 14-16 %) or a chemical desiccant/defoliant is sprayed over the crop. Although it is mainly avoided, during harvest, extraneous material from the ground, including soil and other potential contaminants, are sometimes included in the final seed preparation. The plant material is then threshed inside the harvester to separate the seed from the other material (FSANZ, 2010).

Seed processing involves the receipt of harvested seeds from seed producers through to the supply to growers. In general, the seed can be purchased directly for direct drilling by producers or from special seedling growers. Seed distributors usually receive cleaned/graded seeds from seed processors and are matched to customer requirements including those of leafy green producers (FSANZ, 2010). The seed processing mainly consists of eliminating extraneous material such as soil, weed seeds, insects and other debris. The cleaning usually consists of passing the seeds through a series of sieves and then further sorting via use of a gravity table, where seeds are separated by their weight. The cleaning process may reduce, but is unlikely to eliminate pathogenic microorganisms if present on or in the seed coat (NACMCF, 1999). Once cleaned, seeds are generally packed into bags for the bulk seed market. Seed companies recommend maintaining seed stocks in conventional refrigerators. Seeds should not be frozen. Where refrigerated storage is not available, short shelf-life lines should be stored at temperatures of less than 15 degrees (RijkZwaan, 2005). Some seed suppliers apply the thermocure treatment to lettuce seeds. This treatment improves the germination under high temperature conditions overcoming high temperature dormancy (thermo-dormancy or secondary dormancy), which considerably reduces the problems related to lettuce seed germination (RijkZwaan, 2005).

Leafy greens are usually direct drilled into beds, but recently there has been an increase in transplantation of seedlings. Direct sowing is frequently used because it is cheaper and the plant forms a much better root system by not being limited by a soil pot or a plug cell, but it has also disadvantages such as the loss of uniformity and longer harvest period. Seedlings for transplanting are produced in greenhouses or tunnels. Seedlings for outdoor cropping usually come from nurseries specialising in producing and handling of young plants (Enza Zaden, 2013). Transplanting is often done mechanically in well worked beds. Depending on the crop, the seedlings are transplanted at a specified density. In winter and early spring planting, the crop is usually protected against frost by covering with fleece or plastic.

2.2. Description of production systems for lettuce type

Leafy greens include a wide variety of 'lettuce'-type leafy plants which can be produced in various regions of the world, grown using various agricultural practices, and under different climatic conditions to fulfil the demand both of domestic and export markets (FAO, 2003). Each geographic area is characterized by different soil-type, terrain, hydrological and climatic conditions, cultivar availability or use and cultivation practices. This diversity results in variation within the agricultural production processes in terms of pre-harvest practices, inputs, production volumes, geographical location, environmental conditions, productivity and target markets (FAO, 2003).

Leafy greens can be produced in both open fields and greenhouses. Currently, there is limited information describing the relative proportions of different production systems in the EU but in general about 90% of production takes place in fields, with the remainder occurring in greenhouses. In theory all crops can be produced as hydroponic crops, but cost considerations result in marginal use of this production method (Appendix A, Freshfel, 2013).

2.2.1. Open field production

Leafy greens can be grown in most soil types although best results are obtained on fertile loams that are rich in organic matter. Leafy greens in open fields are usually grown in soil, in a bed system. The bed system utilizes a well-drained soil that will increase temperature faster in the spring and can drain more rain in wet periods throughout the season (Enza Zaden, 2013). Another advantage is that the bed can also be covered with black plastic sheeting for transplanting in plant holes, giving a significantly earlier harvest in colder regions. This plastic will also reduce both soil splash to the leaves and weed problems. Although leafy greens are traditionally cultivated in soil, recently alternative soil-less cultivation techniques have also been used. There are numerous soil-less culture systems available such as New Growing System (NGS^{TM}), the Nutrient Film Technique (NFT^{TM}) system, pot and sac systems, hydroponics, aeroponics and flotation systems (Fallovo et al., 2009; Johnson, 2008; Selma et al., 2012). For example, aquaponics (the integration of aquaculture and hydroponics) is a developing technology where liquid effluent rich in plant nutrients derived from fish manure, decomposing

organic matter, and nitrogenous waste excreted from fish fertilizes hydroponic beds, providing essential elements for plant growth (Fox et al., 2012). Soil-less systems suit short culture cycles and high plant density and have been in particular used for the production of high-value-added crops such as baby and multi-leaves (Nicola et al., 2005). However, leafy greens production in open field under soilless systems is not frequently used and most of these systems have only been used under experimental conditions, as it is the case of aquaponics.

2.2.2. Greenhouse production

In Europe, leafy greens eaten raw as salads (baby and multi-leaf crops as well as whole heads) can be grown in greenhouses. Compared with open field systems, greenhouse production affords protected cultivation and usually leads to an increased yield whilst reducing the impact of climatic conditions. Because of the introduction of greenhouse production systems, many products originally grown in the south of Europe are now also produced in northern countries (EC/SCF, 2002). Compared to the open field systems, the protected culture systems offer many advantages, for example, protection from winds and other adverse weather conditions, such as rain and hail, a reduction in evapotranspiration rate, an increase in photosynthesis rate, and decrease in the harvest period. The covering material of the greenhouses allows an increase in the internal air temperature, and leads to reduced air and soil temperature differences (Nicola et al., 2009). Although many advantages have been attributed to the use of greenhouses to produce leafy greens, it is known that greenhouse leafy greens are usually more susceptible to pests and mechanical damage. However, Goñi et al. (2013) reported that greenhouse lettuce heads had higher nutritional and sensory quality at harvest and lower enzymatic browning than open field grown lettuce heads. As in open field systems, soil and soil-less cultivation can be found in greenhouses.

2.2.3. Other types of production

There is a range of other production systems dependent on the species of plant and it is beyond the scope of this Opinion to consider all cultivation methods. Of the plant species commonly consumed (Appendix A, Freshfel, 2013), cabbage, certain types of chicory (Belgian endive), leafy brassicas and watercress differ substantially to that previously described in this section. However methods of processing as well as risk factors during cultivation, processing, distribution, at retail, catering and in domestic environments have many factors in common.

Cabbage, Chinese cabbage and kale are shallow rooted plants generally grown for their densely leaved heads (cabbage and Chinese cabbage), or open leaves (kale and other leafy brassicas). The leaves are produced during the first year of their biennial cycle which although often cooked, fermented or preserved in a number of different ways, can also be eaten raw. Cultivation is usually by direct seeding in open fields, and mature cabbage heads are developed between 70 and 120 days depending on the cultivar and climate, with slightly shorter growing periods for Chinese cabbage and kale. Harvest generally takes place by hand by cutting the stalk just below the bottom leaves with a knife. The outer leaves are trimmed, and any diseased, damaged, or necrotic leaves are removed. Once harvested, cabbage heads can be stored at 0°C (1°C for processing cabbages) with 90 to 95 percent relative humidity and will last for four to six months depending on the cultivar with shorter periods for Chinese cabbage (2-3 months) and kale (10-14 days).

Chicory consists of two species and is cultivated for its leaves, usually eaten raw as salad leaves. Cultivated chicory is generally divided into three types: radicchio usually variegated red, green or white open leaves (*C. intybus*); sugarloaf which appears like romaine lettuce, with tightly packed green leaves (*C. endivia*); and Belgian endive also known as witloof or witlof (*C. intybus*). The latter has a small head of cream-coloured, bitter leaves and is grown completely underground or indoors in the absence of light in order to prevent the leaves from turning green and opening out. Cultivation generally takes about 6-7 months and can take place in open fields or in greenhouses for radicchio and sugarloaf types. For Belgian endive, the production comprises three phases: the growing of the roots in the field, the storage of the roots and the forcing of the heads. In the first phase the plants are thus grown for approximately 6 months outdoors to develop a deep taproot. Then the whole plant is

harvested, trimmed cooled and stored from 15 days to 2 months. During this cold storage, the roots become vernalized and flower induction is initiated. Finally, the roots are traditionally placed indoors in the soil, or in hydroponic trays, to force the production of a tight bud of leaves. This forcing occurs in the dark and optimally with forced heating at elevated temperatures 16-20°C. This results in the cream-yellowish compact head or chicon from the root which is ready to be harvested after a forcing period of ca. 3 to 4 weeks. Belgian endive is packed in a box with a lid that excludes light, as exposure to light causes the chicons to turn green and become unmarketable. A shelf-life of 21-28 days can be expected at a temperature of between 0 and 2°C which decreases to ca. 10-14 days if kept at 5°C.

Watercress (*Nasturtium officinale*) is cultivated in shallow gravel lined beds, fed with a constant flow of water, which can be chalk-filtered spring or borehole water. Water temperatures above 25.5°C can cause slow or poor growth. For the cultivation of watercress, a large flow of water is needed to supply other nutrients and protect plants from freezing. Thus, in most cultivation situations re-circulation systems are applied which ensures that that there is uniform water movement throughout the bed for even growth of the crop. Production is outdoors and plants are either grown from seed or through vegetative propagation. The growing time from planting to harvest can be anything from 28 to 70 days depending on the climate. Harvesting is either by hand or harvesting machines and for commercial production, plants are rapidly chilled washed and either sold in bunches or packed into 'washed and ready-to-eat' bags. The shelf life is relatively short and similar to other leafy greens eaten raw. Land Cress (*Barbarea verna*) is another type of leafy green which is mostly produced in Spain and Portugal and cultivation follows similar agricultural practices to those for other leafy greens. Land cress grows best in a cool, moist soil and part shade. This crop is usually used as a substitute for watercress as it is easier to grow.

2.2.4. Water sources and irrigation systems

The need for irrigation depends on the soil type and climatic conditions. Where the soils easily retain water, they are irrigated before and after transplanting, and may not need further irrigation until a few days before harvesting. However, in other types of soils, more frequent irrigation is needed (Enza Zaden, 2013). Water from diverse sources (e.g. collected rainfall, subsurface, surface, or reclaimed water) has been used in the production of leafy greens. Sources of irrigation water can be generally ranked by the microbial contamination hazard (Leifert et al., 2008): in order of increasing risk these are potable or rain water, groundwater from deep wells, groundwater from shallow wells, surface water, and finally raw or inadequately treated wastewater. In Europe, the main water sources are surface waters (river, lake), reservoirs supplied by well water or rain water, well water and potable-quality water particularly in the case of hydroponics (Appendix A, Freshfel, 2013). Reclaimed water, which refers to municipal wastewater and industrial process water that has been treated (Directive 91/271/EEC[12]), is reported by the industry not to be used for leafy green irrigation in the EU. Water treatment of wastewater usually includes primary (sedimentation) and secondary treatments (biological oxidation) as well as more advanced tertiary treatments such as chemical coagulation, filtration and/or disinfection. However, these treatments can vary as there are no standards established at EU level for treatment of municipal wastewater or industrial process water to be used for irrigation.

Water quality used for the application of pesticides is also relevant. Pesticides are usually chemical preparations that are routinely used in the cultivation of fruits and vegetables to control pests, weeds, plant pathogens and spoilage bacteria and fungi (Andrews and Kenerley, 1978). Pesticides that are regularly applied to produce have been considered to be a source of microbial contamination (Guan et al., 2001). There is evidence that human pathogens can survive and grow in pesticide solutions (Guan et al., 2001; Ng et al., 2005) and that their application to the surface of leafy vegetables constitutes a risk, particularly near harvest time (Guan et al., 2001; Izumi et al., 2008).

Many irrigation methods (e.g. drip irrigation, overhead sprinkler, furrow, sub-irrigation systems) can be chosen to maintain a good availability of water for the crop (Nicola et al., 2009). In Europe, the major irrigation systems used in agricultural production are drip or sprinkler irrigation (Appendix A,

[12] Council Directive 91/271/EEC of 21 May 1991 concerning urban waste-water treatment. OJ L 135, 30.5.1991, p. 40-52.

Freshfel, 2013). Sprinkler irrigation offers several advantages over surface irrigation methods, such as higher water use efficiency, better fertilizer application and high yield although it cannot be applied when higher wind velocities occur (Camp et al., 2001 ; Tagar et al., 2012). Furrow irrigation is a surface irrigation system that can be found in small-scale farms because it does not require high investment in equipment. Drip irrigation applies water directly to the root zone of plants and its major advantages over sprinkler and furrow irrigation include: saving of water, increased efficiency of fertilizer use, reduced energy consumption and tolerance of windy conditions. Drip irrigation is also recommended for undulating land (Michael, 2008; Tagar et al., 2012). It has been reported that in England, nearly three-quarters of vegetable crops were irrigated using overhead methods such as sprinkler irrigation while the remainder received drip-irrigation (Knox and Weatherhead, 2005; Monaghan and Hutchison, 2012).

2.2.5. Different types of fertilisation, organic/manure/compost

To optimise the crop quality and production it is advisable to apply fertilizer before transplanting, although this may depend on the soil type. Optimal delivery is to apply the fertilizers between the rows which secures full availability for the plants, increases utilisation and avoids chemical burning of leaves from contact with fertilizer (Enza Zaden, 2013). Fertilization can be done with chemical and/or organic fertilizers. Chemical fertilizers are easy to transport, are used efficiently for growth of the plants and give high yields, but it has been observed that with succeeding crops, the quantity of chemical fertilizers has to be increased because of declining soil fertility. Organic fertilizers are available in different forms such as liquid, powder, granular and pelleted from various sources of organic materials. Treated animal manure and compost from wastes and vegetable residues are sometimes used. Where necessary (e.g. due to heavy rain) fertilization can be applied via the irrigation system, which is known as fertigation. The main difference as compared to normal crop fertilization is that fertilizers are added in soluble forms, in low amounts but at high frequencies (Lucena, 1995).

Composted manure products (including those from all farmed animals such as cattle, poultry, etc.) placed on the market must have undergone treatment to inactivate pathogenic microorganisms as defined in Annex V and processed manure in Annex XI to Regulation (EU) No 142/2011[13]. More generally, application on lands of organic fertilizers, manure, slurries, from all farmed animals, fresh or composted, is regulated locally considering hygienic and environmental risks. This may for instance forbid spreading fresh slurries on land within a year before starting food of non-animal origin production, or impose sufficient distance to protect water resources used for food of non-animal origin irrigation.

2.2.6. Harvesting

Leafy greens are manually or mechanically harvested. Mechanical harvest is faster than hand harvesting, but depending on the crop, can result in significant damage to the produce. Mechanical damage during harvest can become a serious problem, as plant injuries predispose produce to decay, increased water loss as well as increased respiratory and ethylene production rates which can accelerate deterioration and may minimize internalization and proliferation of microbiological contamination (Kitinoja and Kader, 2002). Manual harvesting is still often practiced for whole heads. This means that the product is separated from the plant roots and manually removed from the growth substrate (soil or soil-less) using a knife or clipper. During manual harvesting the stem is cut at ground level and the head trimmed of unusable leaves. This is also known as 'in-field coring', which involves removal of the cores and dirty or damaged wrapper leaves of whole heads during the harvesting process, and in some cases it is also followed by spraying with a solution that may contain disinfectants or anti-browning agents (FAO, 2003; Suslow et al., 2003). Although the process of coring-in-field may reduce the microbial populations, this process exposes internal tissues to the field environment thereby increasing the risk of direct contamination (FAO, 2003). Harvesting and field

[13] Commission Regulation (EU) No 142/2011 of 25 February 2011 implementing Regulation (EC) No 1069/2009 of the European Parliament and of the Council laying down health rules as regards animal by-products and derived products not intended for human consumption and implementing Council Directive 97/78/EC as regards certain samples and items exempt from veterinary checks at the border under that Directive. OJ L 54, 26.2.2011, p.1-254.

packing by hand is usually assisted by a variety of equipment that includes conveyors and mobile packing stations. Plant heads can be wrapped or bagged in plastic film by the cutter or packer (USDA, 2004).

Baby leaves and multi-leaves are more suitable for mechanical harvest than whole heads (Figure 3). In the case of multi-leaves, different leaf crops can be grown and harvested in the same line. Leafy greens that have been harvested during rainy weather or harvested by machine are often contaminated with soil and may be rinsed or sprayed with clean or potable water before grading and packing.

Source: Ana Allende, reprinted with permission of Quality and Safety Lab CEBAS-CSIC

Figure 3: Mechanical harvesting of baby spinach.

2.2.7. Cooling

The handling conditions between the harvest and the processing of leafy greens are critical to maintain the quality and safety of the product and should be done as soon as possible after harvest (ASHRAE, 1998). Although not always applied by the growers, guidelines recommend that leafy greens are cooled as soon as possible after harvest by either forced-air cooling, vacuum cooling (iceberg lettuce) or spray-vacuum cooling (leaf lettuce/leafy greens, romaine lettuce, spring mix, spinach), also designated hydrovac cooling (Thompson et al., 2007).

All mechanical damage should be minimized and, whenever required, the raw material should be refrigerated as soon as possible after harvest (Rogers et al., 2006). If the temperature of the product is not immediately reduced after harvest it will affect the quality of the product due to (i) the maintenance of high respiration and metabolism rates usually associated with rapid consumption of sugars, acids, vitamins and other constituents (ii) a high weight loss and (iii) an increase in development of decay. Delays in the cooling will also cause water and texture losses in leafy greens (Thompson et al., 2001). Additionally, temperature is the most important factor to restrict growth of foodborne bacterial pathogens if leafy greens become contaminated.

The best temperature to maintain the quality of leafy greens eaten raw as salad is between 0 and 5ºC, but these products are often kept at 10 to 12ºC in the display cabinets or even at room temperature (Oliveira et al., 2010a). In the case of leafy greens, different cooling systems have been recommended such as the use of cold rooms, forced-air cooling, vacuum cooling and hydro vacuum-cooling (Thompson et al., 2007). The use of conventional cold rooms is common during winter, when the temperature of the crops is usually low. The use of forced-air cooling reduces the temperature of the product very rapidly as the cold air flows through the boxes allowing direct contact between the cold air and the vegetable product. Vacuum cooling is one of the most effective methods based on the evaporation of some of the product's water at low atmospheric pressures. It has been shown that the shelf-life of minimally processed vegetable products is improved when the product is cooled using vacuum cooling compared to forced-air cooling (Rogers et al., 2006). Vegetable products with a high surface area per unit weight are suitable for vacuum cooling, one of the most commonly used cooling systems in commercial production. To reduce the water loss due to the water evaporation, spray water

is used (water spray vacuum cooling or hydrovac), which increases the surface humidity of the tissue before or during the vacuum process. A modification of this system is the use of a perforated plastic film over the moist product during the vacuum procedure (Isik, 2006).

2.3. Description of EU leafy greens sector

Information provided by Freshfel (Appendix A and B) shows the wide diversity of practices in the EU, at all stages of production, including, as indicated in the above sections, drip or sprinkler irrigation, cultivation in various substrates (soil, artificial, hydroponic) and settings (open air, greenhouse, tunnels or production rooms). A wide range of packaging and storage conditions are used within this industry. Products may be packed or loose and stored refrigerated or un-refrigerated. Only leafy greens for the fresh cut industry follow a well defined process. However there is limited information available and it is not possible to assess the relative proportions of commercial packaging and storage conditions used at the EU level. Quantitative data are available for some Member States, but it must be stressed that the situation differs considerably between Member States. Despite these limitations, the general features of leafy greens production in EU can be summarized as follows.

The main species produced in EU are *Lactuca sativa*, *Cichorium endivia*, *Beta vulgaris*, *Valerianella locusta*, *Cichorium intybus*, *Eruca vesicaria* subsp. *sativa*, *Spinacea oleracea*, *Brassica rapa*, *Brassica oleracea* and *Nasturtium officinale*. Apart for *Spinacea oleracea* (spinach), *Cichorium intybus* (Belgian endive) and *Brassica* spp. (cabbage), these leafy greens are mostly consumed fresh-cut and raw. The amounts produced for these leafy greens species differ widely. Although annual data for some Member States are not available, between 2007 and 2012, the EU production of lettuce only can be estimated to between approximately 2 million and 2.5 million metric tons per year (Table 6, Appendix B), with import representing only 2000 to 5000 metric tons per year. For a comparison, the annual production of Belgian endive (hydroponically grown *Cichorium intybus*) in EU is around 280 000 metric tons per year. Therefore, there is a wide variation in the production volumes of the different commodities. The majority of the production is whole head with, for example, this comprising 85% of leafy green production in France. There are no data that permit an assessment of the share of small scale versus large scale producers. Most (estimated to be 90%) leafy greens are produced in fields with the remaining production in greenhouses, except Belgian endive which is always grown in production houses. Production takes place all over the EU depending on the season, with the main producers being Spain, Italy, France and Germany. Irrigation is mostly via drip and sprinkler, from surface water, reservoirs or wells. Most fresh leafy greens are sold unprocessed, e.g. around 70% in France and in the Netherlands with the rest being sold fresh-cut. Raw material for fresh-cut processing is usually harvested manually, except for lamb's lettuce (*Valerianella locusta*) and baby leaves. Processing includes grading, cutting, (manually or mechanically), cleaning, rinsing and drying (mechanically), packing (manually or mechanically).

Good hygienic practices guidelines for leafy greens (both raw and fresh cut) exist at national level, and companies' specifications (e.g. Global Good Agricultural Practices (GAP)) at a broader level. Fresh-cut companies must be registered as food processing establishments.

Some measures to reduce the risk of contamination of leafy greens during primary production are implemented. In addition, manure is frequently composted, chiefly to inactivate weeds, pests and plant pathogens, but this should also reduce human foodborne pathogens. For the same reasons, soil may be treated e.g. by steam or sunlight. Some operators use chemical disinfection of irrigation water. However the percentage of the leafy greens production affected by these measures is not known. For fresh-cut leafy greens, the incoming processing water is of drinking (potable) quality, and disinfectants (chlorine, peracetic acid, ozone) are added during processing in some Member States, depending on national regulations, to maintain the hygienic quality of the processing water. The cold chain for fresh-cut leafy greens is maintained from just after harvest of the raw material to the end product at retail, at temperatures that depend on national legislation.

2.4. Comparison of production systems and practices between the US and EU

A comparison was obtained between United States and EU production practices (Appendix A, Freshfel, 2013). Production in the US is generally concentrated in the South West of the US with consequential longer transport times and shelf lives (14 to 18 days shelf-life in the US as compared with 7 to 11 days in the EU). The scale of production is generally larger in the US than in the EU. In the US processing facilities are generally near to the production site, whereas these are more often located nearer to the consumer market in the EU. There is larger market penetration of fresh cut product in the US as compared to the EU. However with the available data it was not possible to establish if these differences contributed to elevated risks for contamination by foodborne pathogens (specifically *Salmonella* and Norovirus) which resulted in differences in reporting of infections associated with the consumption of leafy greens.

It should be taken into account that production practices are also diverse among the different countries within EU. For instance, in the South-East of Spain, the scale of production is larger than in any of the other areas of EU, although it is still smaller than that in the South-West of the USA. Thus, apart from scale and proximity to the consumer market, agricultural practices in the South of Europe are not dissimilar to those applied in US.

3. Risk factors for microbiological contamination during agricultural production

Production practices, growth conditions and the location of the edible part during growth (soil, soil surface, aerial part) in combination with intrinsic, extrinsic, harvesting and processing factors will affect the microbial status of leafy greens at the time of consumption (EC/SCF, 2002). The great variability in the production systems and associated environments of leafy greens can lead to a wide range of unintentional or intentional inputs that are potential sources of food safety hazards (Suslow et al., 2003). It has been shown that microbial food safety hazards and sources of contamination vary considerably from one type of crop production to another and from one particular setting/context to another, even for the same crop (FAO, 2003). Also, bacterial distribution on or in the plants themselves may differ according to the route of exposure although there are few studies which focus on the effect of the contamination route on pathogen colonization or internalisation (Mitra et al., 2009; Park et al., 2012). The following sections are intended to identify and characterize potential risk factors for contamination of leafy greens.

3.1. Environmental factors

Environmental factors refer to the specific conditions of the primary production area, climate, type of crop which might have an impact on the safety of the leafy greens (CAC, 2003). Several review studies have focused on the microbial contamination routes and persistence of pathogens in produce fields (Beuchat, 2006; Critzer and Doyle, 2010; Doyle and Erickson, 2008; Franz and van Bruggen, 2008; Liu et al., 2013; Olaimat and Holley, 2012; Pachepsky et al., 2011; Park et al., 2012). The available research studies have mostly focused on the impact of contaminated soil, the use of fertilizers, irrigation water sources, quality and frequency, and climate change on pathogen prevalence and concentration (Franz et al., 2005; Ge et al., 2012; Hutchison et al., 2008; Islam et al., 2004a; Islam et al., 2004c; Liu et al., 2013; Natvig et al., 2002; Semenov et al., 2007).

3.1.1. Factors linked to the adherence, survival and internalisation of pathogens with leafy green plants

There are also studies which evaluated the impact of produce type and cultivars on the colonization of pathogens (Barak et al., 2008; Park et al., 2012). Hutchison et al. (2008) reported that the numbers of *Salmonella* recovered from lettuce were higher than those recovered from spinach. The association between microbial contamination and plant age has also been evaluated (Park et al., 2012). For instance, Bernstein et al. (2007b) showed significantly higher levels of contamination with *Salmonella* on mature rather than on young lettuce plants. However, (Brandl and Amundson, 2008), reported higher population of *Salmonella enterica* on young leaves than on middle leaves harvested from mature romaine lettuce heads, suggesting that leaf age may affect pre-harvest as well as on farm post-

harvest colonization. The moist conditions between the folded inner leaves may contribute to the survival of enteric pathogens in the lettuce head (Van der Linden et al., 2013a).

The survival of *Salmonella enterica* for two years on butterhead lettuce seeds and their subsequent survival and growth on the seedlings has been demonstrated (Van der Linden et al., 2013b). Furthermore, another study using an artificial thale cress system reported the occasional presence of *Salmonella* Newport in seeds and chaff harvested from contaminated plants, depending on the method of inoculation (Cooley et al., 2003). These authors suggested that contamination of the seed occurred directly from contaminated chaff or by invasion of the flower or silique. However, there are no reports that under natural conditions, contamination of leafy greens by *Salmonella* originates from contaminated seeds.

The adhesion of *Salmonella* Enteritidis to crispy-type lettuce leaves was also evaluated in the context of competitive microflora (Lima et al., 2013). They found that higher numbers of endogenous microorganisms on lettuce leaves reduced *S.* Enteritidis adhesion. Some experimental studies have reported that survival of *Salmonella* in lettuce during growing is relatively short (4-8 days) (Van der Linden et al., 2013a). However, it also has been reported that *Salmonella* persisted for up to 63 days and 231 days on lettuce and parsley, respectively (Islam et al., 2004b). Berger et al. (2009) reported that *S.* Typhimurium, *S.* Enteritidis and *S.* Senftenberg adhered efficiently to leafy vegetables whereas other *Salmonella* serovars (*S.* Arizona, *S.* Heidelberg and *S.* Agona) did not. Furthermore, the mechanisms for adherence differed between serovars (Barak et al., 2005; Barak et al., 2007; Gibson et al., 2006; Lapidot and Yaron, 2009). The role of plant host factors (plant morphology, age, cultivar, water content, inhibitory phenolics and mesophyll thickness) are also likely to be important (Yadav et al., 2005). There have been very few studies on the effect of different plant cultivars on survival of *Salmonella* spp.. For instance, Klerks et al. (2007) demonstrated differential interaction between cultivars of commercially available lettuce cultivars (Tamburo, Nelly and Cancan) and survival of *S. enterica* serovars. In this case, the evaluated *S. enterica* serovars were each able to colonize soil-grown lettuce epiphytically, but only *S. enterica* serovar Dublin was also able to colonize the plants endophytically.

The internalization of *Salmonella* spp. within the vegetable tissue of leafy greens has been demonstrated (Park et al., 2012). *Salmonella* Typhimurium is capable of penetrating the epidermis of iceberg lettuce leaves through open stomata in a process that involve flagella motility and chemotaxis (Kroupitski et al., 2009). The role of flagella in *Salmonella* leaf attachment has been further investigated showing that different *Salmonella* serovars use strain-specific mechanisms to attach to different salad leaves such as lettuce, rocket and spinach (Berger et al., 2009). Several studies have demonstrated the internalisation of *S.* Typhimurium in leafy greens harvested following cultivation on contaminated manure-amended soil and irrigation water (Bernstein et al., 2007a; Franz et al., 2007; Ongeng et al., 2011; Pachepsky et al., 2011). However, various factors have been shown to affect the ability of human pathogens to internalize, including: growth substrate (soil vs. hydroponic solution), plant developmental stage, pathogen genus and/or strain, inoculum level, and plant species and cultivar (Hirneisen et al., 2012). Golberg et al. (2011) demonstrated that internalisation of *Salmonella* Typhimurium via the leaf epidermis is variable in leafy greens. They observed that the highest incidence of internalisation was in iceberg lettuce and arugula leaves, while romaine and red-lettuce showed significantly lower incidence. Few studies have evaluated whether physical damage of produce leaves or roots influences the fate of pathogens (Park et al., 2012). Root removal of romaine lettuce increased the number of *Salmonella* Newport cells in lettuce leaves (Bernstein et al., 2007a). Human Norovirus RNA was detected in lettuce leaves after exposure of the roots to the virus particles (Di Caprio et al., 2012).

Internalization in leafy greens has been observed after artificial inoculation of high levels of *Salmonella* making it difficult to assess its importance under natural conditions (Warriner and Namvar, 2010). For instance, Golberg et al. (2011) applied 10^8/ml *Salmonella* Typhimurium on leaves to observe internalization though the epidermis of various leafy greens, Franz et al. (2007) added 10^7/ml or 10^9/ml *Salmonella* Typhimurium to growth solution or soil to observe internalization

through the roots. Internalization through the roots of cabbage was observed with 10^7 *Salmonella* Typhimurium /g of soil, but not with 10^4/g (Ongeng et al., 2011). In leaves of basil grown in soil with 10^6/g *Salmonella* Newport, internalized *Salmonella* were mostly detected only after enrichment (Gorbatsevich et al., 2013), indicating a low transfer rate from root to leaves. The survival of internalised *Salmonella* has rarely been studied. In basil leaves, internalized *Salmonella* Newport could not be recovered after 22 h (Gorbatsevich et al., 2013). In lettuce, *Salmonella* Newport was found internally 2 days after inoculation of roots, but not after 5 days (Bernstein et al., 2007b).

Internalisation of Norovirus, or surrogate viruses, in plant tissues has been observed in several experimental studies. Cut pieces (1 cm^2) of lettuce can take up murine Norovirus when placed in suspensions of approximately 4 x 10^4 murine Norovirus pfu ml^{-1} (Wei et al., 2010); after incubation at 4ºC for 5 min, approximately 10^3 pfu could be detected. Growing lettuce hydroponically in nutrient solution spiked with 5 x 10^8 genome equivalents[14] murine Norovirus to mimic a single gross contamination event (nutrient solution replaced after one day) resulted in leaves containing approximately 10^4 PCRU per 50 mg; when constant contamination was mimicked by growing plants in nutrient solution spiked with 5 x 10^5 PCRU murine Norovirus and replacing the spiked solution each day, the level of internalisation was lower at approximately 2 logs PCRU per 50 mg leaf tissue (Wei et al., 2011). Delivering the spiked nutrient solution from beneath by capillary force of the growing plant in soil resulted in leaves containing approximately 10^2 PCRU virus per 50 mg leaf tissue (Wei et al., 2011). Murine Norovirus and hepatitis A virus can also be internalised in spinach and green onion tissues after the plants were grown in nutrient medium or soil substrate spiked with several logs PCRU of the virus (Hirneisen and Kniel, 2013), the virus remaining infectious for at least 5 days after internalisation with no decline in infectivity. These results demonstrate the possibility that viruses may become located in plant tissue following exposure via contaminated soil or irrigation water. Transpiration may have a role in virus uptake, which may be enhanced by increasing humidity (Wei et al., 2011). However, the virus levels used in experimental studies may be higher than those which could be encountered during crop production; furthermore, information on Norovirus internalisation gained through use of surrogates should be interpreted with caution, as properties of different viruses may affect uptake into, or clearance from, plants. For example, when lettuce plants growing in soil under outdoor conditions were exposed to 10^9 – 10^{10} PCRU human Norovirus or canine calicivirus in irrigation water delivered from below the roots, human Norovirus was not subsequently found in the edible portions of the plants, although canine calicivirus was detected in vascular liquid (Urbanucci et al., 2009).

3.1.2. Conditions in the field and adjacent land

The conditions at the growing field play a vital role in the microbial safety of leafy greens. Each farm environment (including open field or greenhouse production) should be evaluated independently as it represents a unique combination of numerous characteristics that can influence occurrence and persistence of pathogens in or near fields of leafy greens (Strawn et al., 2013a). The Codex code of hygienic practice for fresh fruits and vegetables establishes that primary production should not be carried out in areas where the known or presumptive presence of pathogens would lead to an unacceptable likelihood of transfer to horticultural crops intended for human consumption (CAC, 1969, 2003). If vegetables are grown next to an animal-rearing operation, there is a potential for the product to become contaminated, directly or indirectly, by animals, run-off, bioaerosols, dust or vectors associated with the animal operation such as birds, rodents or flies (Brandl, 2006; FAO, 2003; Gelting et al., 2011). Although this did not involve *Salmonella*, these risk factors are illustrated by two *E. coli* O157 outbreaks linked to leafy greens, in which the outbreak strains were isolated from cattle near to the fields producing the incriminated leafy green (Jay et al., 2007; Soderstrom et al., 2008).

[14] In this study, a most probable number approach was followed using end-point detection of RTPCR signal in dilutions of nucleic acid extracted from the sample, and the data were expressed as 'PCR-detectable units' (PCRU). However, in this Opinion any such data will be expressed as 'genome equivalents' on the supposition that the lowest PCRU may represent amplification of one target RNA molecule, and to facilitate a harmonised comparison of findings of different studies. It should be noted however that due to the lack of culturable NoV (and consequently well-established reference materials), detection and quantification limits may differ depending upon the exact experimental conditions used in the cited works.

Although declining, *Salmonella* may be persistent in the soil for extended periods of time (Holley et al., 2006; Islam et al., 2004a; Islam et al., 2004b; Natvig et al., 2002). Recently, Strawn et al. (2013) identified soil properties and topographic features as constraints on *Salmonella* occurrence in produce fields because not all croplands had an equal risk of contamination. Soil characteristics and topographic variables corresponding to the proximity of sampled areas to other landscape types, including imperviousness of surfaces, water or pasture were identified as factors for predicting locations containing pathogens. Additionally, *Salmonella* survival has been shown to increase in moist soils (Chandler and Craven, 1980; Holley et al., 2006).

3.1.3. Climatic conditions

Climate conditions have been related to changing disaster risk patterns mainly by the increase in frequency and intensity of extreme events (Solomon et al., 2007). It has been reported that climate changes will mainly impact on the contamination sources and pathways of pathogens onto leafy greens during the pre-harvest phase (Liu et al., 2013). Recently, several reviews have addressed the impact of climate change on leafy greens (FAO, 2003; Liu et al., 2013; Tirado et al., 2010). Climate change has been identified as having the potential to increase pathogen contamination of food and water. Variation has been observed in levels of pathogens in agricultural land and water with extreme weather events such as alternating periods of floods and droughts (Liu et al., 2013; Rose et al., 2001; Tirado et al., 2010).

Rainfall increases the risk of splashing manure and soil particles onto lettuce in proportion to the amount and force of precipitation (Cevallos-Cevallos et al., 2012; Franz et al., 2005; Girardin et al., 2005; Liu et al., 2013). An increase in frequency and severity of extreme precipitation events may lead to contamination of soil, agricultural land, ground or surface water and leafy greens with pathogens originating from sewage which derive from agricultural, urban, or industrial settings (Solomon et al., 2007). Because of compaction, heavy rainfall after drought can result in more severe run-off which might be an intermediate contamination pathway of pathogens from manure at livestock farms and from grazing pastures and release of large numbers of faecal coliforms and a variety of pathogenic microorganisms, into the environment including the growing area of crops and into water courses (Abu-Ashour and Lee, 2000; Donnison and Ross, 2009; Guber et al., 2006; Orozco et al., 2008; Parker et al., 2010). Faecal contamination of agricultural soils has been shown to increase after flooding (Casteel et al., 2006). After flooding, lettuces have been contaminated with *Salmonella* spp. although contamination was rapidly reduced in the product, probably due to the climatic conditions and high total UV radiation after the flooding event (Castro-Ibañez et al., 2013).

Increased temperature can increase the rate of microbial growth. It may also influence the population of insects and pests found in and around farms that are capable of transferring human pathogens to leafy vegetables. However, increased UV from sunlight may result in a decrease in potential human pathogens in soil and on both the stems and leaves of leafy greens (Tannock and Smith, 1972; Zaafrane et al., 2004). Several studies highlighted the positive relationship between temperature and rainfall and the number of salmonellosis cases (Semenza and Menne, 2009; Zhang et al., 2010). However, the mechanisms underlying the observed seasonality in foodborne disease are not fully understood, but are likely to involve a complex interplay of multiple factors (Liu et al., 2013). Relative humidity (RH) has been shown to have an effect on survival of human pathogens on plant surfaces (Dreux et al., 2007). In general, it has been reported that warm temperatures and high humidity facilitate the survival or growth of pathogens on produce (Park et al., 2012). The correlation between dust as a carrier of microorganisms and the spread of contaminants has been demonstrated (Davies and Wray, 1996; Varma et al., 2003). The spread of contaminants through aerosols is also well documented (Baertsch et al., 2007).

3.1.4. Contact with animal reservoirs

Domestic animals such as cattle, sheep, chickens, dogs, cats and horses can contaminate crops with faeces if they pass through growing areas. However, while domestic animals may be separated from growing operations, it can be more difficult to control access by wild animals (e.g. frogs, lizards,

snakes, rodents, badgers, foxes, deer or wild boar) and birds (Harris et al., 2003; Lowell et al., 2010). *Salmonella* has been isolated with varying frequencies from various species of wild animals that can come into contact with leafy green production, including wild boar (Vieira-Pinto et al., 2011; Zottola et al., 2013), deer, birds (Benskin et al., 2009; Carlson et al., 2011; Lawson et al., 2010; Ramos et al., 2010), rabbits (Vieira-Pinto et al., 2011), rats (Lapuz et al., 2008) and flies (Pava-Ripoll et al., 2012). Wildlife has been suggested as a cause of contamination of the food production and processing chains with *Salmonella* (Hilbert et al., 2012), but this has very rarely been confirmed microbiologically for leafy greens. An example is given by Sagoo et al. (2003b) who reported isolation of *Salmonella* Umbilo (a serovar rarely found in humans) from outbreak cases, the incriminated rocket salad, and the lizards from the rocket growing fields. In another outbreak linked to baby spinach, the outbreak strain of *E. coli* O157 was found in patients, spinach, feral swine and cattle (Jay et al., 2007). In this example, the primary reservoir was presumably cattle, and the investigation indicated that feral swine probably transmitted the pathogen to leafy greens. Contact with animal reservoirs may also occur after harvest, for instance in open storage facilities or packing sheds (Jalava et al., 2006; Munnoch et al., 2009), although this has not been reported for *Salmonella* and leafy greens.

3.2. Organic amendments (manure, slurries, composts, wastewater treatment, sludge and sewage)

Organic fertilizers such as animal manure may introduce faecal pathogenic bacteria, viruses and parasites to leafy greens if manure is not adequately aged or otherwise treated before application (Mawdsley et al., 1995; Strawn et al., 2013b). Additionally, manure piles stored next to growing operations may represent a risk of contamination via run-off, vertebrate and insect vectors, dust or aerosols (Brandl, 2006; James, 2006; Suslow et al., 2003). The prevalence of a range of foodborne pathogens in animal wastes (slurries and manure) from livestock in the UK has been reported (Hutchison et al., 2004). *Salmonella* was detected in 5% to 18% of samples, depending on the animal species. The positive samples contained an average of around 10^3 CFU/g with maximal values of 10^6-10^7 CFU/g. Numbers of *Salmonella* in positive samples of pig farm manure treatment units in the EU and in the US, were between 0.4 to 4 $\log_{10}$ MPN/100ml, from influx of raw material to the secondary treatment pond (McLaughlin and Brooks, 2009). In the UK and in France, these authors reported respectively 3/58 and 6/50 pig manure treatment units were positives for *Salmonella*. In the UK, frequencies of *Salmonella*-positive samples were similar for fresh manure or for manure sampled after periods of on-farm storage. Using fresh or inadequately composted livestock wastes in production of fresh produce is therefore a risk factor for *Salmonella* (Hutchison et al., 2004).

Composting of organic wastes can reduce the number of *Salmonella* initially present by several $\log_{10}$ units, provided that an adequate combination of temperature increase, retention time and relative humidity are achieved (Ceustermans et al., 2007; Lung et al., 2001). In addition, it has been reported that *Salmonella* will not grow in composted cow manure if recontaminated (Kim and Jiang, 2010). Therefore, using adequately composted wastes in production of leafy greens should not represent a risk factor for *Salmonella* contamination. However, waste digestion at mesophilic temperatures cannot consistently reduce the number of foodborne pathogenic bacteria initially present (EFSA, 2007b; Lung et al., 2001), and material processed in such ways represents a risk factor.

No *Salmonella* outbreaks linked to consumption of leafy greens consumption have been traced to the use of contaminated manure. However, manure is normally applied several weeks before harvest and is unlikely to be available during outbreak investigations. Transmission to leafy greens of *Salmonella* from manure or organic wastes applied to soil has been measured experimentally. For example, *Salmonella* was detected from some samples of rocket grown in cow manure amended soil inoculated with 10^4 - 10^5 CFU *Salmonella*/g, and harvested 17 weeks after manure application (Natvig et al., 2002). Similarly, *Salmonella* was detected on some spinach leaves planted in manure amended soil containing 10^6 CFU *Salmonella*/g, up to 21 days after planting (Arthurson et al., 2011). These experiments were done in artificial conditions, mimicking, to some extent, the outdoor climate. Ongeng et al. (2011) used a non-virulent strain of *Salmonella* to inoculate manure used to fertilize outdoor cabbages. *Salmonella* was detected on cabbage leaves 120 days after manure application, but

only for the highest level of *Salmonella* inoculated (10^7 CFU/g of manure). *Salmonella* declined when inoculated in soil amended manure, at rates that varied according to climate, and both the nature of the soil and the manure (Garcia et al., 2010; Natvig et al., 2002; Ongeng et al., 2011; Semenov et al., 2009). The risk of finding *Salmonella* on leafy greens grown on soil amended with contaminated manure decreases with the time between manure application and harvest.

Some studies have associated the extent of contamination and the type of animal waste applied (Park et al., 2012). Islam et al. (2004a) showed that pathogen survival was greater in produce grown in soil amended with composted poultry manure than in manure from cattle. Similarly Nyberg et al. 2010 found a longer survival for *Salmonella* in soil amended with poultry manure than with cattle manure. When evaluating the effects of cattle feeding regime on the fate of *Salmonella enterica* it was found that the roughage type was not associated with the survival of the pathogen in plants grown in soil amended with that cattle manure (Franz et al., 2005). Recent multivariate analysis showed that manure application within a year increased the likelihood of a *Salmonella*-positive field, whilst the presence of a buffer zone around the crop had a protective effect. Further significant risk factors were irrigation (within 3 days prior to sample collection), reported wildlife observation (within 3 days prior to sample collection), and soil cultivation (within 7 days prior to sample collection) which all increased the likelihood of a *L. monocytogenes*-positive field (Strawn et al., 2013b).

Lower eukaryotic organisms (particularly nematode worms) may act as a temporary reservoir for some foodborne pathogens (including *Salmonella*) in the soil. This property may provide a risk of contamination in the preharvest environment by increasing the dispersal and survival of pathogens. Kenney et al. (2005) showed that *S.* Newport and *S.* Poona could remain in the gut of the nematode *Caenorhabditis elegans* for at least two generations and transfer these bacteria to the guts of wild type worms. Furthermore, the thermotolerant nematode *Diploscapter* which is able to survive in turkey manure as well as *C. elegans* was shown to be able to be colonised by *S.* Poona, and shed *Salmonella* into soil amended with composted turkey manure (Anderson et al., 2006; Gibbs et al., 2005). Caldwell et al. (2003) showed that *S.* Newport in the gut of *Caenorhabditis elegans* was afforded some protection against the effects of sanitizers in both in vitro models as well as on the surface of iceberg lettuce leaves but not on the surface of cantaloupe melons.

The risk of sewage or wastewater contaminating vegetables with human foodborne pathogens, including Norovirus and *Salmonella*, has been reviewed (Bryan, 1977). Norovirus is excreted in high numbers in faeces by infected humans (EFSA Panel on Biological Hazards (BIOHAZ), 2012), and the virus is likely to be present in wastewater, sewage and wastewater treatment plant effluent, in particular during periods of the year with high incidence of disease in the human population. For instance, in Ireland, the concentration of Norovirus in wastewater entering a sewage treatment plant was between 10^4 and 10^6 genome equivalents[15] per litre (Flannery et al., 2012). Monitoring of Norovirus over a period of one year in a wastewater treatment plant in Sweden showed that the activated sludge contained between less than 10^4 and $2x10^5$ genome equivalents of Norovirus per litre, with incoming wastewater containing between 10^4 and 10^7 genome equivalents of Norovirus per litre (Nordgren et al., 2009). Murine Norovirus (a frequently used surrogate for human Norovirus) declined during anaerobic digestion of a pig slurry (at least 4 $\log_{10}$ cycles over 13 days), at both mesophilic and thermophilic temperatures (Baert et al., 2010). Among sewage-sludge treatments used in practice (6 days duration), only thermophilic processes inactivated pathogenic viruses other than Norovirus (Rotavirus and Enterovirus) (Spillmann et al., 1987). The fate of viruses from sewage sludge or sewage effluents after application to soil used for vegetable production was investigated for enteroviruses (Tierney et al., 1977) in outdoor experimental plots. The population of infectious enteroviruses declined in the soil but some samples of lettuce planted just after or just before sewage application, were contaminated with these viruses at harvest. Most studies on Norovirus and vegetable crops identified the risk of repeated contamination events following the use of irrigation with

[15] The term 'genome copies' has been used in some publications to describe data obtained using a calibrated quantitative RT-PCR as a detection assay. However it is possible that RNA fragments containing the primer sequences can be detected and therefore 'genome equivalents' is used in this Opinion.

insufficiently treated reclaimed or surface waters that become contaminated via discharges of untreated or insufficiently treated municipal wastewater (see section 3.3.) and a short duration between contamination and harvest. Application of sewage sludge to soil used to cultivate leafy greens is a risk factor for Norovirus contamination, as for other pathogenic viruses. However, such applications are rarely used for leafy green production in EU (Appendix A, Freshfel, 2013).

3.3. Water use during production

Contaminated water may serve as a source of microorganisms entering the food chain, however adequate supply of water is critical, particularly at pre-harvest and on farm post-harvest stages where it is used for irrigation, application of pesticides, cleaning of equipment, washing produce, etc. (FAO, 2003). The role of contaminated irrigation water in the external and/or internal contamination of leafy greens has been reviewed and it is cited as a major potential risk factor (Brandl, 2006; Doyle and Erickson, 2008; Gil et al., 2013b; Hanning et al., 2009; Pachepsky et al., 2011; Sapers et al., 2006; Suslow, 2010; Suslow et al., 2003). However, there is limited evidence from outbreak investigations clearly identifying irrigation water as the source of contamination of leafy greens with foodborne pathogens. In one outbreak caused by *E. coli* O157, irrigation water was the most likely cause, but the outbreak strain was not recovered from the water (Soderstrom et al., 2008). Contamination of water with pathogens may be transient, making them difficult to detect during outbreak investigation which can take place some time after the cases were exposed.

Different irrigation strategies (overhead sprays, drip irrigation systems or flooding of fields through furrows) differ in their potential for spread of microbial contamination. In FAO/WHO (2008) it was agreed that subsurface irrigation lowers the risk of pathogen transfer from water to growing plants. Enteric bacteria and viruses aerosolized in spray irrigation systems have been shown to travel considerable distances (Teltsch and Katzenelson, 1978). The delivery of irrigation water through overhead systems can clearly result in extensive contamination of the production environment (FAO, 2003). In a green house experiment, the relationship between levels of *Salmonella* in irrigation water and presence of *Salmonella* on spray irrigated parsley was assessed (Kisluk and Yaron, 2012). A minimum contamination level of 300 cfu/ml of irrigation water was needed to detect *Salmonella* on parsley leaves after enrichment. When higher levels of *Salmonella* were present in the irrigation water, similar numbers of *Salmonella* were found per g of parsley leaves as per ml of water.

Many studies have shown a relationship between increased precipitation accompanied by runoff or discharge of untreated wastewater and increased concentration of faecal indicator organisms or pathogens in water (Dorner et al., 2007; Ferguson et al., 1996; Shehane et al., 2005). In the United States, the highest *E. coli* concentrations in river water were found during periods of greater rainfall intensity. (Schilling et al., 2009) Consequently, following heavy rainfall, the use of irrigation water is more likely to result in product contaminated with pathogens (Castillo et al., 2004; Ensink et al., 2007; Okafo et al., 2003).

Because of the time between irrigation and harvest, pathogenic bacteria can be reduced in numbers by UV radiation from sunlight, drying, or competition with commensal microbiota (Brandl and Amundson, 2008; Ottoson et al., 2011). Increasing the interval between the time of contamination and the point of harvest significantly decreased the likelihood that pathogenic and non-pathogenic strains of *E. coli* would be present in the harvested product (Fonseca et al., 2011; Moyne et al., 2011).

Even if direct contact between irrigation water and the aerial, edible parts of leafy greens is avoided, irrigation water may contaminate the soil or substrate, where the bacteria can survive for some time. For example, following irrigation of greenhouse substrates mixed with water contaminated with *Salmonella* Newport (around 10^6 cfu/g substrate) the number of bacteria was reduced by less than a factor of 10 after approximately 2 weeks and were still detectable after 70 days in the substrate (Bernstein et al., 2007a). Furthermore, although declining, *Salmonella* Typhimurium introduced via contaminated irrigation water (10^5 cfu/ml) in natural soil, in open field conditions, was still detected

200 days after irrigation and survived to the same extent when introduced via contaminated manure or contaminated irrigation water (Islam et al., 2004c).

Contaminated water used to prepare pesticide solutions may also represent a risk. *Salmonella* can survive or multiply in some pesticide formulations and transfer from pesticide water to iceberg lettuce (Guan et al., 2001; Stine et al., 2011). Norovirus is also likely to survive in pesticide-containing water (Verhaelen et al., 2013b).

Norovirus can be found in surface water, for instance the highest concentration found in the Meuse river in the Netherlands in a winter season was 1700 genome equivalents[16]/L (Westrell et al., 2006). In a wastewater treatment plant in Sweden, the outgoing water contained in average 1.5 $\log_{10}$ less noroviruses than the incoming wastewater (Nordgren et al., 2009), a reduction not sufficient to eliminate Norovirus: over a two year period, the outgoing water contained between 10^4 and 10^6 genome equivalents of Norovirus. In Ireland, an average reduction of 0.8-0.9 $\log_{10}$ genome equivalents of Norovirus was observed over a one year period in water samples from a wastewater treatment plant (Flannery et al., 2012). In a study of leafy green primary production sites in three European countries, Kokkinos et al. (2012) detected Norovirus GI in 1/35 samples of irrigation water and GII in 1/25 samples.

As previously stated in section 5.2 of the Scientific Opinion of the EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards (BIOHAZ), 2011a) it is important to estimate the concentration of Norovirus (as well as *Salmonella*) on leafy greens after over-head irrigation, and to assess the volume of retained water on such products as a function of the duration of irrigation and the extent of pathogen adherence to leafy greens needs to be determined. However, although the current PCR-based technology is able to measure rates of decline for Norovirus on leaf surfaces after irrigation, these do not necessarily correlate with the decline in infectivity (see section 11.1).

3.4. Equipment

Contamination of leafy greens can occur at any point in the farm-to-plate continuum. However, handling by field workers and contact with equipment make the field production stage of particularly high risk for contamination by foodborne pathogens (Yang et al., 2012). Whole heads of leafy greens can be subject to trimming and in-field coring. This is a relatively recent practice and it has been designed to increase processing plant production yields from traditional levels of 60-70% to nearly 100% by removing wrapper leaves and outer leaves in the field and harvesting only ready to process leafy greens (Anonymous, 2001). This is common in the US but there are no data available about the extent of this practice in Europe.

Manually cutting in the field can transmit and disseminate contamination. McEvoy et al. (2009) and Taormina et al. (2009) demonstrated that a single coring knife artificially inoculated with *E. coli* O157:H7 could successively contaminate up to nineteen iceberg lettuce heads. Factors influencing pathogen transfer from soil to iceberg lettuce via contaminated coring knife blade included water content of clay and sandy soils, inoculum concentration, and degree of blade contact with the edible tissue (Yang et al., 2012). When comparing the tools used for in-field coring it has been shown that the cutting blade has a higher potential than the coring ring to be contaminated by soil, but less opportunity to transfer pathogens to lettuce during harvesting (Yang et al., 2012).

The equipment used for mechanical harvest has also been identified as a potential source of contamination. The harvesting machine could pick up faecal deposits in the field, contaminating large volumes of product, as suspected in the *E. coli* O157 outbreak linked to baby spinach (Jay et al., 2007). Other equipment that could represent a source of contamination are containers/boxes and conveyor belts as suggested by previous research studies (Johnston et al., 2006; Prazak et al., 2002). These and other studies confirm the importance of hygiene and equipment sanitation. However, more

[16] See footnote 14 above.

research is needed to determine the specific contributions of different types of equipment to possible cross-contamination and growth of pathogens (FAO, 2003).

Cooling of leafy greens involves rapid removal of field heat in harvested produce prior to long-term storage (FAO, 2003). Most of the growers use conventional cold rooms but some quicker systems which will remove heat from the produce are used. One of the most commonly used technologies is vacuum cooling. However two potential risks have been associated to this technology. Firstly water is sprayed to avoid moisture loss due to evaporation from the leafy greens (FAO, 2003) and secondly, the use of negative pressure vacuum cooling was found to significantly increase the infiltration of artificially inoculated *E. coli* O157:H7 into lettuce tissue (Li et al., 2008). Vacuum cooling changes the structure of lettuce tissue, such as stomata, suggesting a possible mechanism of internalization by pathogens.

3.5. Worker health and hygiene, worker training

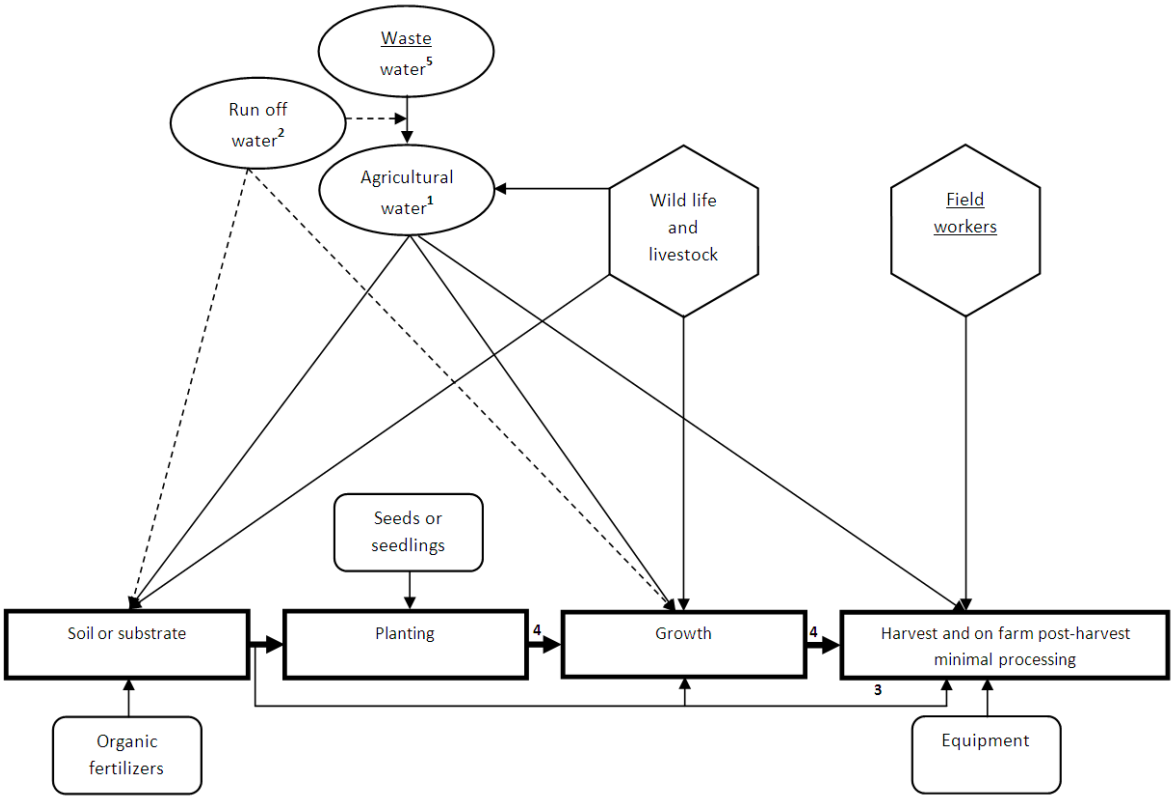
People working with leafy greens eaten raw as salads can transfer microorganisms of significant public health concern by direct contact (Gil et al., 2013b) and contamination will be influenced by hygiene practices as well as land preparation and methods of harvest (James, 2006). For example, provision of instructions on the proper use of gloves or hand washing facilities is necessary to prevent the transfer of pathogens to leafy greens (Suslow et al., 2003). Additionally, leakage from portable toilets to fields and in-field defecation has also been identified as potential source of contamination (Suslow et al., 2003).

As previously stated in section 4.4.4 of the Scientific Opinion of the EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards (BIOHAZ), 2011a) the most common food worker errors identified in relation to outbreaks due to Norovirus are ready-to-eat food handling by an infected person or carrier of the virus and failure to properly wash their hands (Todd et al., 2007). Poor personal hygiene was also identified as a contributing factor in outbreaks of gastroenteritis where Norovirus was assigned as the causative agent (Noda et al., 2008). Another study showed that asymptomatic food handlers at later stages of the food chain tested positive for Norovirus GII.4 strain in Japan (Ozawa et al., 2007): the number of virus shed by symptomatic and asymptomatic food handlers was similar. It has been estimated that approximately 16% of the population are asymptomatically infected, and shed Norovirus without being aware (Amar et al., 2007; Phillips et al., 2010). Norovirus can also be shed for several days after symptoms have resolved (Atmar et al., 2008; Zelner et al., 2013), and presymptomatic shedding can also occur (Atmar et al., 2008; Lo et al., 1994). This capacity for Norovirus to be shed in the absence of symptoms is a significant factor underlying the hazard of these highly contagious viruses, and clearly indicates the absolute necessity for hand hygiene at all times by all food handlers.

Cross-contamination via food handlers' gloves could also be a factor: one study found that approximately 5% of murine Norovirus spiked onto iceberg lettuce could be transferred to the fingertips of nitrile gloves after touching the produce for 5 seconds (Verhaelen et al., 2013a).

3.6. Conclusion

A summary of the risk factors for microbiological contamination during agricultural production is presented in Figure 4.



1: Irrigation, pesticide solutions, washing the harvested product.
2: Transfer of pathogens, from animal faeces, livestock, waste water, contaminated soil, to leafy greens can be increased by flooding, run off water or heavy rains.
3: Contamination of leafy green by pathogens present in soil can be increased at harvest (e.g. during mechanical harvesting).
4: Attachment to and internalisation of the pathogens to leafy green tissues (if possible in real production conditions).
5: Insufficient reduction of pathogens by waste water treatment.

Figure 4: Summary of the main risk factors (ellipses) during primary production and harvest of leafy greens. The relative importance of the risk factors is not illustrated in the figure. Underlined risk factors are particularly relevant for Norovirus. Full lines refer to possible contamination pathways of leafy greens from different sources and the dotted lines refer to the possibility to increase this contamination. The thicker lines refer to the production flowchart of leafy greens and the thin lines refer to possible contamination pathways of leafy greens from different sources.

The main risk factors for the contamination of leafy greens with *Salmonella* are diverse and include:

- Environmental factors, in particular proximity to animal rearing operations, seasonality and associated climatic conditions (e.g. heavy rainfall causing floods) that increase the transfer of pathogens from their reservoirs
- Contact with animal reservoir (domestic or wild life)
- Use of untreated or insufficiently treated manure or compost
- Use of contaminated agricultural water (for irrigation or pesticides treatments)
- Cross-contamination by food handlers and equipment at harvest or on farm post-harvest

Salmonella tends to decline on the surface of leafy greens during primary production. Therefore contamination events close to harvest (e.g. irrigation water, floods), at harvest (e.g. by food handlers) or on farm post-harvest (e.g. cross-contamination via water or from equipment or by food handlers) are the most important risk factors at primary production.

The main risk factors for the contamination of leafy greens with Norovirus at primary production are diverse and include:

- Environmental factors, in particular climatic conditions (e.g. heavy rainfall or floods) that increase the transfer of Norovirus from sewage or sewage effluents to irrigation water sources or fields of leafy greens)
- Use of agricultural water for irrigation or pesticides treatments contaminated by sewage
- Cross-contamination by food handlers and equipment at harvest or on farm post-harvest.

Norovirus can persist on leafy greens.

4. Description of processing methods for leafy greens

Leafy greens may be further processed to obtain ready-to-eat products, and these steps include: selection, elimination of external leaves, cutting, washing, rinsing, dewatering, packaging and storage. Other types of processing (e.g. freezing, mashing and unpasteurized juicing, blending etc) are either never or very rarely used with leafy greens and are not further considered in this Opinion. Some of these products are subject to cooking, pickling and other processes but these are outside the scope of this Opinion.

In general, the first step is the reception and inspection of the raw material to assure the rejection of inferior quality product particularly that which has undergone mechanical damage which may minimize internalization and proliferation of microbiological contamination. Following this selection, high quality product is stored under refrigeration conditions, and processing will vary depending on the type of product. For whole heads, external leaves and the core are removed by hand. Hand knives and stationary coring units are used for this operation (Gil and Selma, 2006). The other parts of the lettuce are shredded to pieces of about 4-6 cm in size, using industrial rotary stainless steel blades. The temperature in the processing plant is usually between 5 to 10ºC. When baby leaves or multi-leaves are processed, steps such as the elimination of external leaves and cores are not needed, and in most instances, these types of products start their processing in the pre-washing or washing step.

Thorough washing and cooling of fresh-cut leafy greens immediately after cutting are important steps in fresh-cut processing. In most processing lines, the product immediately drops into a washing tank after shredding. Washing can be achieved by simply spraying with potable water, although it generally involves the immersion of the product in chilled water (1 to 10ºC). Disinfectants are sometimes added to the water in baths or wash-tanks depending upon national policies for their use and approval for the use of disinfectants.

The terms disinfectants and disinfection agents are used to define substances that are applied to non-living objects to destroy microorganisms, although they do not kill all microorganisms, especially spores and cysts. Therefore, decontamination agents that are applied to maintain the microbial quality of the process wash-water are defined as disinfectants. USEPA (United States Environmental Protection Agency) however defines sanitizers as a decontamination agent that reduces microorganisms on food contact surfaces by at least 99.999 %. Although both terms can generally be used synonymously, for the decontamination of process wash water or fresh produce such as leafy greens, described in this Opinion, the term disinfection agent or disinfectant will be used for those decontamination agents applied to process wash water to avoid cross-contamination. Sanitizer or

sanitizing agent will be applied to those decontaminating agents applied to reduce the level of microorganisms on leafy greens.

Modern aeration 'jacuzzi' washing systems generally consist of three separate washing stages and three tanks. The first of these tanks aims to eliminate general field dirt and debris. The microbial quality of the water can be maintained with the use of a disinfection agent, which will avoid cross-contamination between different lots. If disinfectant is not used, processing of leafy greens relies on continuous addition and refreshing of washing baths with large volumes of potable water, up to 40 l/kg of raw produce, to minimize the accumulation of microorganisms in the water and transfer of microorganisms from the water to the fresh-cut leaves. (Olmez and Kretzschmar, 2009; Selma et al., 2008; VMM, 2006). In some instances, the pre-wash is done with showers to avoid accumulation of organic matter in the process water. Product is then usually immersed in a second tank in which the water may be treated with a disinfection agent to prevent cross-contamination during washing (FAO, 2003) if permitted by national regulations (FAO, 2003). Whenever disinfectants are used, the last stage before packaging should be the rinsing step which requires very low doses of disinfection agent to maintain the hygienic quality of the water. Leafy greens can also be sprayed with potable water for this last rinsing step. For leafy greens which float, a washing system where high volumes of air are blown into the tank through pipes located just beneath the surface of the water is a currently used method (Artés and Allende, 2005; Simons and Sanguansri, 1997). This creates a vigorous 'Jacuzzi' effect, which causes produce to tumble around and creates the mechanical action needed for optimal cleaning (Gil et al., 2013b). Maintenance of water quality is a key factor to avoid risks of cross-contamination.

There are three parameters that have to be controlled in washing fresh-cut products: quantity of the water used, temperature of the water and, if used, the concentration of disinfectant (Yildiz, 1994). Dirt and debris that sinks to the bottom of the tank can be released through a periodic drainage system with on-going renewal by fresh water. In some cases, leafy greens can be subjected to heat shock treatments, in particular for visual quality to prevent enzymatic browning. The effects of these treatments on food borne pathogens is not well understood but may be limited by the need to avoid heat damage of the leaves. Microbial inactivation is not the purpose of this treatment. Heat transfer or time and temperature of the treatment are limited and difficult to control uniformity among the washed fresh-cut product. This is thus not a microbial reduction or leafy green safety intervention strategy and it is still necessary to combine this heat-shock treatment with a disinfection agent to guarantee the microbiological quality of the process wash water.

After washing, 'dewaterers', centrifuges, screens and dehumidifiers are used to remove excess water. The dewatering method used in most of the fresh-cut processing lines is centrifugation (Gil and Selma, 2006). The time and speed of centrifugation, or alternative dewatering systems, are key parameters to be adjusted for each product. To reduce tissue damage and consequent microbial deterioration in leafy greens that are too delicate to withstand centrifugation, forced air or air-bed conveyors are recommended (Turatti, 2011) and these are widely used in Europe.

The final operation in the processing of fresh-cut leafy greens takes place in the assembly and packaging room. Virtually all fresh-cut leafy greens are refrigerated under modified atmosphere packaging to achieve the required commercial shelf life. In the assembly room, after inserting the correct amount of product into the packages, the packs are sealed. Polymeric films are used in an effort to maintain product quality, while extending shelf-life (Gil and Selma, 2006). Before sealing, the atmospheres within the packages may be evacuated or flushed with a mixture of gases to establish more rapidly a desirable modified atmosphere. Atmospheres with low pO_2 (0.2–0.5) combined with pCO_2 (4.0–6.0) at the steady-state preserved lettuce quality by the control of browning and the prevention of off-odours and off-flavours (Martínez-Sánchez et al., 2011).

Proper temperature control of storage and transportation is critical to maintaining visual quality, crispiness and to delay microbial growth during the shelf-life for fresh-cut leafy greens. Thus, the storage unit must maintain the fresh leafy vegetables at appropriate temperatures which may differ

between Member States, types of product, packaging, and the expected shelf-life (AFDO, 2004; Wright, 2004). Temperature and humidity information can be tracked to determine if food products are transported and stored under appropriate conditions (Matthews, 2009). Recent studies have demonstrated that exposure of leafy greens to low relative humidity (RH) conditions before washing decreased internalization of *Salmonella* spp. compared to internalization in baby spinach exposed to high RH (Gomez-Lopez et al., 2013). The effect of cooling on the persistence of human Norovirus GGIi on iceberg lettuce was studied by Mormann et al. (2010): neither viral capsid integrity nor genome copy number was significantly reduced by storage at 6ºC for 2 days. In general, low temperatures are conducive to virus survival (Rzeżutka and Cook, 2004) it is likely that, if anything, lowering the temperature of leafy greens will enhance the potential for contaminating Norovirus to remain infectious. The recommended marketing temperature for fresh-cut leafy greens eaten raw as salad is 7ºC although operators will apply lower temperatures to optimize quality and shelf life (Appendix A, Freshfel, 2013), however these products may occasionally be abused at higher temperatures (10 to 12ºC) that sometimes occur for example in display cabinets (Oliveira et al., 2010a).

5. Risk factors for microbiological contamination during processing treatments, including the main processing practices

Processing leafy greens into fresh-cut products increases the risk of bacterial growth and contamination by breaking the natural exterior barrier of the produce. The degree of processing and handling common to many fresh-cut processing operations can provide opportunities for contamination and for spreading contamination through a large volume of product (IFT/FDA, 2001). The most relevant risk factors during processing are environmental factors, water sources, worker health and hygiene and equipment.

5.1. Environmental factors

Environmental factors refer to the specific conditions of the processing area, which might have an impact on the safety of the leafy greens (CAC, 2003). The environment of the processing plant may represent a risk for contamination. For instance, at the beginning of the fresh cut salad industry in EU in the 80s, *L. monocytogenes* was found more frequently in the processed product than in the raw materials used by the processing plant (Velani and Roberts, 1991). Temperature is also a key factor in fresh-cut processing plants. Many research papers described the relevance of low temperature as a strategy to avoid/reduce bacterial growth of foodborne pathogens in leafy greens (Abadias et al., 2012; Oliveira et al., 2010a; Posada-Izquierdo et al., 2013; Sant'Ana et al., 2012). In general, it is reported that the growth of *Salmonella* in leafy greens can be controlled by ensuring that these products are stored at a temperature below 7ºC. Oliveira et al. (2010a) observed that the population of *Salmonella* decreased in shredded romaine lettuce approximately 1 log unit after 10 days at 5ºC, while it increased about 2 log units after 3 days at 25ºC.

Leaf internalization of *Salmonella* and viruses pre-harvest was discussed in section 3.1. Internalisation of *Salmonella* in the detached leaves can also occur due to the impact of on farm post-harvest handling conditions. Recently, Gomez-Lopez et al. (2013) demonstrated that humidity during on farm post-harvest handling affects the internalisation of *Salmonella enterica*. Exposure of leaves to low relative humidity conditions before washing, which reduced the tissue water content, decreased internalisation of *Salmonella* compared to high relative humidity. However, *Salmonella* internalisation was unaffected by the illumination conditions (Gómez-López et al., 2013). Survival of *Salmonella* can occur on leafy greens and, under certain conditions of storage, growth may occur especially on fresh-cut leafy greens, although most of the available literature focuses on the potential growth of *Salmonella* in fresh-cut leafy greens (Franz et al., 2010; Puerta-Gomez et al., 2013; Sant'Ana et al., 2012; Sant'Ana et al., 2013).

5.2. Water sources (washing)

Water use during processing of leafy greens has been identified as a potentially important source for cross-contamination with faecal indicator organisms (e.g. *E. coli*) and human enteric pathogens (Allende et al., 2008; Buchholz et al., 2012; Holvoet et al., 2014a; Holvoet et al., 2012; Luo et al., 2011; Rodriguez-Lazaro et al., 2012; Shen et al., 2013). Washing and disinfection have economic and environmental implications. It is assumed that if produce is washed without the use of sanitizers, larger quantities of water are required than in the presence of sanitizers. Sanitizers and their concentrations as well as the mode of washing vary depending on the processor. As an example chlorine at 40-60 mg free chlorine per litre may be used when washing tanks or fluming are used. In this case the temperature of the water is usually maintained between 4-10ºC, contact times are 1 to 2 min and pH values between 6 and 7.5 to ensure the presence of chlorine in the hypochlorous acid form and minimize corrosion of equipment (FAO, 2003; Van Haute et al., 2013).

However, it should be noted that process wash water in the washing tank can serve as a source of cross-contamination and may result in the build-up of microorganisms, from the crop which may include pathogens (Allende et al., 2008). Quantitative data on lettuce contamination and cross-contamination were established in a simulation study by Holvoet et al. (2014a). This showed that only a small proportion (<1.5%) of the microorganisms (whether *Escherichia coli*, *E. coli* O157, MS2 phage or murine Norovirus) was transferred from the water phase to lettuce, although it highlights the vulnerability of leafy greens to cross-contamination by enteric bacteria and viruses during the washing stage. Therefore, many studies have focused on the maintenance of water quality during washing as it is now specified by many authors that 'antimicrobial chemicals, when used appropriately with adequate water quality, help to minimize the potential microbial contamination of processing water and subsequent cross-contamination of the product' (FDA, 2008; Lopez-Galvez et al., 2009). Thus, sanitizing agents are recommended to be used to maintain the hygienic quality of the water and prevent cross-contamination of the product, in spite of their limited direct antimicrobial effect on microbes attached to the produce (Gil et al., 2009). The efficiency of sodium hypochlorite (NaOCl) and peroxyacetic acid (PAA) to reduce murine Norovirus 1 (MNV-1), a surrogate for human Norovirus, was investigated by Baert et al. (2009b). The study showed that 5 min. exposure to 200 mg/litre NaOCl or 250 mg/litre PAA in the washing water accomplished an additional reduction of 1 log of MNV-1 on shredded iceberg lettuce (compared with washing in water without disinfectants). The effectiveness of NaOCl was greatly influenced by the presence of organic material, which was not observed for PAA. In a prior study Baert et al. (2008) studied the inactivation of MNV-1 in spinach processing water and noted that 5 min of exposure to 20 ppm of PAA resulted in a 2.41 log reduction of MNV-1 plaque forming units in the processing water itself (although no decrease in number of MNV-1 genomic copies by RT-qPCR detection was noticed). These studies illustrate the potential of PAA and NaOCl in reducing the likelihood of cross-contamination during the washing process and establishing a minor reduction of MNV-1, as a surrogate virus for human Norovirus, present on leafy greens. Additionally, if the wash water contains pathogens, they may be internalised in the tissues (Gomez-Lopez et al., 2013). Although many studies have assessed the ability of crops to internalize human bacterial pathogens such as *Salmonella* through root uptake (Hirneisen et al., 2012), internalisation at the wounded tissue of fresh-cut leafy greens during washing steps in processing has not been extensively studied. In general it has been proposed that washing of leafy greens in water colder than the produce (negative temperature differential) increases internalisation of bacteria, due to a contraction of the gases present in intercellular spaces (Bartz and Showalter, 1981). However, in a recent study where *Salmonella* was able to internalise during washing of baby spinach, there was no statistically significant effect of a negative temperature differential between the baby spinach and the water (Gomez-Lopez et al., 2013).

5.3. Equipment

It has been reported that conveyor belts, centrifugation and filling operations are not usually significant sources of contamination (Garg et al., 1990). However, other studies found that numbers of natural microbiota (total count) increased about 1 log unit CFU/g after centrifugation (Allende et al., 2004), and surfaces of processing equipment have been recognized as sources of microbial

contamination and recontamination (Lehto et al., 2011). However, it should be taken into account that *Salmonella* is very rare on leafy greens (see Section 9, Tables 1 and 2) therefore it is highly unlikely that it will be found on processing equipment. One of the main concerns is the ability of bacteria to form biofilms. These are difficult to remove with the cleaning practices routinely used, and there is potential for them to persist in the processing plant and act as a resident source of contamination (Romanova et al., 2007). In an outbreak investigation, the equipment used for cutting and shredding lettuce in a commercial setting was identified as the source of contamination confirming that poorly cleaned and maintained equipment can harbour microorganisms, including pathogens, and provide a reservoir of contamination (Stafford et al., 2002). For microorganisms other than *Salmonella,* shredders and cutters may carry a high microbial load (Lehto et al., 2011) and their use may increase total bacterial counts obtained from the processed leafy greens (Garg et al. 1990). These have been historically implicated as the source of cross-contamination of shredded cabbage with *L. monocytogenes* (Lainé and Michard, 1988). Additionally, in a microbial sampling study of fresh-cut produce processing companies in Belgium, *E. coli* was not detected on the food handlers' hands/or gloves but was found on conveyor belts and weighing units, highlighting these as potential sources of cross-contamination (Holvoet et al., 2012). Using *E. coli* O157:H7 experimentally inoculated on leafy greens, Buchholz et al. (2012) showed that 90% of the inoculum was shed to the disinfectant-free water, with this pathogen also contaminating the surfaces of shredders, conveyor, flume tank, shaker table and dewatering centrifuge. These examples highlight equipment as a potential source of cross-contamination. This is also presumably the case for *Salmonella*, although this has not been reported. The possibility for virus contamination of produce items to spread via cross-contamination through contact with food processing or preparation surfaces also exists (Escudero et al., 2012) although unlike bacterial contaminants multiplication of viruses outside the host cannot occur.

5.4. Worker health and hygiene, worker training

As for any other sectors processing ready-to-eat foods, lack of compliance of workers with Good Manufacturing Practices (GMP) and Good Hygiene Practices (GHP) is a risk factor for leafy green processing. Good practice includes adequate training as well as both hand washing and toilet facilities which are further considered in Section 12.1.5. The contamination of shredded lettuce with *Shigella* by a food handler, caused a widespread outbreak in the US (Davis et al., 1988) and illustrates this possibility, although nothing similar has been reported for *Salmonella* or Norovirus and leafy greens. However, since the excretion of Norovirus by ill persons can be very high (EFSA Panel on Biological Hazards (BIOHAZ), 2012) this represents a risk factor for contamination.

5.5. Conclusion

During processing, contamination or cross-contamination via equipment, water or by food handlers are the main risk factors for contamination of leafy greens for both *Salmonella* and Norovirus.

Submersion of fresh-cut leafy greens during processing in washing tanks presents a risk of contamination from the water circulating in the washing tank water. For *Salmonella,* this risk is reduced if disinfectants are properly used within the washing tank. There are few studies with surrogate viruses, such as Murine Norvirus, that investigate the effectiveness of chemical inactivation of Norovirus in processing water. The effectiveness of chlorine against Norovirus is not fully defined due to the lack of an infectivity assay, although studies observing the effect of chlorination on detectable viral RNA (Shin and Sobsey, 2008) indicate that chlorine concentrations used to treat potable water would be effective. Adherence or biofilm formation of *Salmonella* on equipment may become a source of contamination for leafy greens during processing and may be difficult to remove by routine cleaning methods.

6. Description of the distribution, retail and catering including domestic and commercial environments for leafy greens

Distribution of leafy greens represents very diverse practices however this usually involves several steps of transport, storage, packaging and handling. Transport and distribution can be done at chilled

or ambient temperature, in a variety of packaging formats and units, depending on the type of product, the region and the season. Distribution of leafy greens is done via various retails outlets ranging from large supermarkets, to small shops or public markets, for both packaged and loose products. Leafy greens are also sold as raw cut product in salad bars at both retail and in catering, often allowing for self selection and service by the consumer. Washing of product may take place in a similar manner to that outlined in primary processing (see Section 4), but is more likely to be in sinks with running potable water used for general food handling. Some use of water to regenerate product may also take place.

In hotels, restaurants or catering establishments, leafy greens can be prepared on-site starting from intact harvested leafy greens supplied directly from the farmer, auction or the wholesale market, or the caterers may purchase fresh cut leafy greens from wholesalers or fresh-cut processing plants. In contrast to processing plants which are mainly dedicated to fresh-cut produce (although some other ingredients may be added to the fresh-cut leafy green package), catering establishments handle or prepare a wide variety of foodstuffs. Hygiene practices in caterers in EU are also very diverse. For instance, in the UK, Sagoo et al. (2003a) found that salad vegetables were only displayed at chill temperatures (below 8°C) in two thirds of the establishments surveyed; specific serving utensils were used by only one third of these establishments while use of bare hands to handle salad was observed in another third.

7. Risk factors for microbiological contamination during distribution, retail and catering including domestic and commercial environments

The primary risk factors for contamination of leafy greens during distribution, retail and catering are cross-contamination through direct or indirect contact with contaminated water, equipment or handling by infected persons.

7.1. Water sources (washing)

Water which has been contaminated with bacteria and viruses, and is then used in food preparation, can cause contamination of leafy greens. This represents a similar contamination or cross-contamination risk as occurs during processing (see Section 5.2). It has been shown that viruses can be transferred from contaminated water to the surfaces of berry fruit and leafy greens (Rodriguez-Lazaro et al., 2012).

7.2. Equipment

There is the possibility for virus contamination from various food products to spread via cross-contamination through contact with food processing or preparation surfaces. For example, this could occur through cutting of a contaminated item followed by using the same utensil to cut uncontaminated items without adequately cleaning them first (Escudero et al., 2012; Wang et al., 2013). When environmental swabs were taken from surfaces, in kitchens as well as staff facilities during the Norovirus high season (February-March) the virus was detected in 21/374 (6%) catering companies and 37/233 (16%) institutional settings (Verhoef et al., 2013).

Due to the wide diversity of foodstuffs potentially prepared and handled in catering establishments, cross-contamination with foodstuff more frequently contaminated with *Salmonella* than leafy greens is a risk factor. In particular, the prevalence of *Salmonella* on leafy greens is estimated to be lower than for some types of raw meat, in particular pork or poultry meat. For instance in the US an outbreak of *Salmonella* Montevideo was presumably due to cross-contamination between chopped cilantro and raw chicken meat (Patel et al., 2010). The same risk of cross-contamination may exist at retail for unpackaged leafy greens, although this has not been documented, probably because of adequate segregation between leafy greens and fresh meat at retail.

7.3. Worker health and hygiene

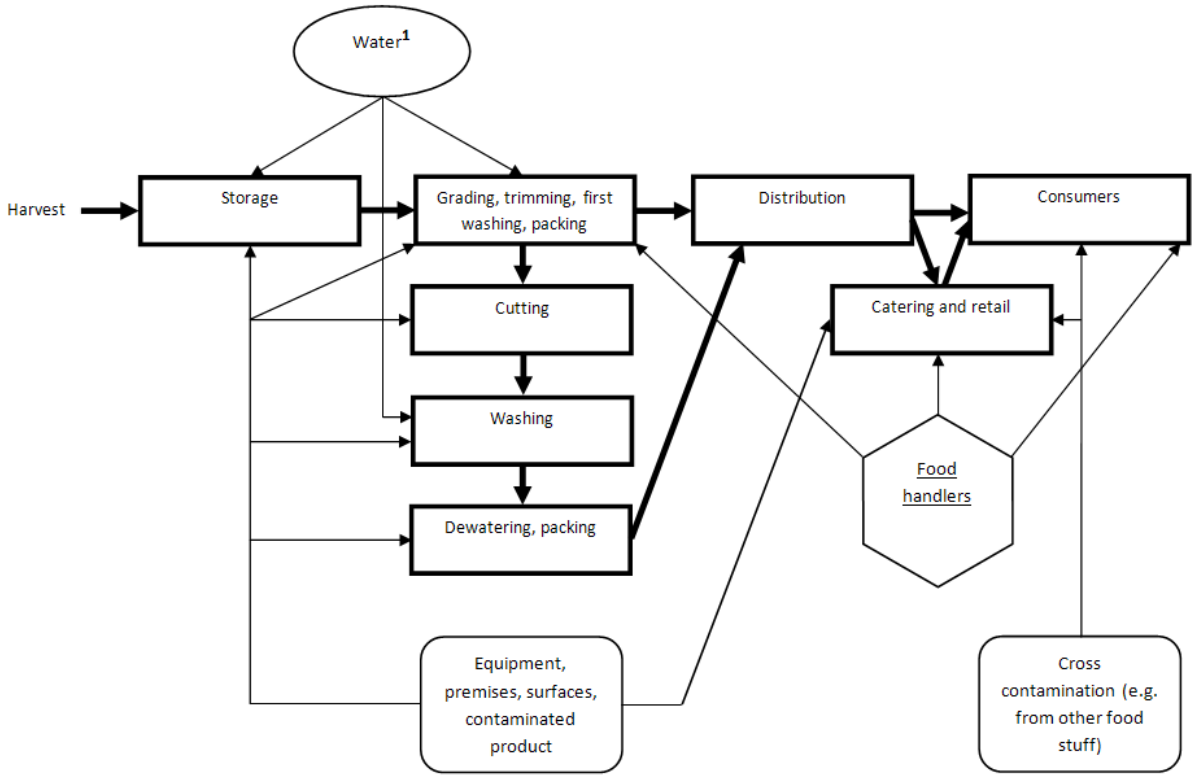
Contamination of leafy greens with Norovirus can occur through contact with the hands of infected persons during preparation. It is possible for a proportion of the viruses contaminating a human hand or fingertip to be transferred to a food surface (Bidawid et al., 2000). Cross-contamination via food handlers' gloves could also be a factor (Verhaelen et al., 2013a). Poor hand hygiene, e.g. not washing thoroughly following the use of toilet facilities and prior to handling of foodstuffs, is an important risk factor for contamination of food.
In a study in the Netherlands involving face-to-face interviews with food handlers working in catering companies and institutional kitchens several gaps in food hygiene education and training were identified. For example, there was little awareness of the transmissiveness of Norovirus and 8 to 11% of the food handlers would not be sent home after reporting gastroenteritis symptoms (Verhoef et al., 2013). In 17 of 40 (42.5%), reported foodborne or waterborne Norovirus outbreaks in Belgium during the period 2000-2007, a food handler was implicated as the origin of the outbreak (Baert et al., 2009a). In eight of these 17 outbreaks, a sick food handler or food handler with a recent history of gastroenteritis was observed. One outbreak in the United States identified three separate clusters of cases associated with a caterer, where one of the food handlers, with a history of gastroenteritis, was involved in preparing three separate catered meals at work (Payne et al., 2006). In 2007, 10 NoV foodborne outbreaks were reported affecting 392 persons in Belgium and in the majority of these outbreaks food handlers in the food service operation preparing sandwiches or meals were the suspected cause (Baert et al., 2009a).

Risk factors for leafy greens in salad bars will include the potential for cross-contamination between products and utensils as well as from poor food handler and consumer hygiene. There is also the possibility of malicious contamination which has the potential to cause large outbreaks (Torok et al., 1997).

In one *Salmonella* Enteritidis outbreak attributed to consumption of lettuce in a restaurant, the investigation suspected contamination by a food handler with diarrhoea, although this was not supported by microbiological evidence (Severi et al., 2012). Although less documented than for Norovirus, contamination of leafy greens with *Salmonella* by food handlers is a potential risk.

7.4. Conclusion

A summary of the risk factors for microbiological contamination after harvest of leafy greens is presented in Figure 5.



1: At processing step, input water must of drinkable quality but it can be contaminated during processing (e.g. by the incoming leafy green) and disseminates the pathogens.

Figure 5: Summary of the main risk factors (ellipses) after harvest of leafy greens. The relative importance of the risk factors is not illustrated in the figure. Underlined risk factors are particularly relevant for Norovirus. The thicker lines refer to the production flowchart of leafy greens and the thin lines refer to possible contamination pathways of leafy greens from different sources.

At distribution, retail, catering, in domestic and commercial environments, cross-contamination of items, in particular via direct or indirect contact between raw contaminated food of animal origin and leafy greens are the main risk factors for *Salmonella.* These cross-contamination risks include the environments of salad bars.

At distribution, retail, catering and in domestic or commercial environments, the Norovirus-infected food handler is the main risk factor. This can be direct or indirect via poor hand hygiene or food contact surfaces that have been subjected to cross-contamination. These contamination and cross-contamination risks include environments of salad bars.

The use of contaminated water for washing of leafy greens or utensils, slicing equipment or working benches are other risk factors for both *Salmonella* and Norovirus. For *Salmonella* growth of the pathogen could occur whenever leafy greens (in particular the fresh-cut leafy greens and probably to a lesser extent the intact whole heads or leaves) are not stored at chilled temperature for a prolonged period, provided relative humidity is sufficient.

8. Analytical methods for the detection and enumeration of *Salmonella* in leafy greens

8.1. Standardisation of methods for detection and enumeration of *Salmonella* in leafy greens

Methods for detection of *Salmonella* spp. in FoNAO are well developed and analytical reference methods standardised and widely adopted across laboratories testing food, including that for Official

Control: EN/ISO standard method 6579[17] is prescribed in Regulation 2073/2005[18] when analysing pre-cut ready-to-eat fruit and vegetables in the scope of the verification of compliance with the currently established food safety microbiological criterion for *Salmonella* spp.

Alternative methods based on modifications of the ISO method using alternative enrichment media or isolation media (chromogenic media) or using immunoassays and real time PCR are also available for rapid detection of *Salmonella* in leafy greens. Many of these methods have been ISO 16140 validated showing performance characteristics equivalent to the EN/ISO standard method 6579. If *Salmonella* positive results are obtained by use of immunoassays or real time PCR based assays it is recommended that these results are confirmed by isolation of *Salmonella* colonies.

9. Data on occurrence and levels of *Salmonella* on leafy greens

The presence of the infectious hazards in FoNAO is usually the effect of a series of adverse and uncommon contamination events. Data on the total number of samples investigated as well as the total number of positive samples for *Salmonella* spp. reported in FoNAO as part of EFSA's Zoonoses web-based reporting from 2004 to 2011 showed a prevalence of 0.48%. Thus the overall prevalence of *Salmonella* spp. on leafy greens is assumed to be low (< 1%) (EFSA Panel on Biological Hazards (BIOHAZ), 2013).

Several studies were conducted on leafy vegetables (whole crops or fresh-cut) either sampled at farm level, in fresh-cut processing companies or in distribution or retail establishments in different countries and on different continents.

Tables 1 and 2 show a summary of the occurrence of *Salmonella* in whole and fresh-cut leafy greens available in the literature.

In most instances, pathogen contamination of leafy greens is considered a 'rare' event (Table 1), so direct pathogen screening is likely to be ineffective.

Most of the studies mentioned in Tables 1 and 2 used the ISO 6579:2002[19] classical culture method for detection of *Salmonella* on leafy greens. Alternatively, some of the prevalence studies in Table 1 or 2 used other standard methods and some of these have equivalence to the ISO 6579. These methods may differ in the use of an alternative enrichment medium, selective isolation medium, identification method or in the use of a prior (validated) rapid screening method before starting isolation of *Salmonella*. Overall, detection methods for *Salmonella* are well established and have a long track record of comparative results, common use and experience in place in laboratories worldwide. Standardization in analysis for *Salmonella* is recommended not only for the selection of the detection method but also with regard to sampling and sample preparation protocols. The latter is probably not critical for fresh-cut bagged vegetables or for sampling at retail but, there are no widely used guidelines or consensus on appropriate sampling location or sampling method within a leafy greens processing company or in the field at primary production. Neither does part 4 of ISO 6887-4:2003[20] include guidelines on sample preparation in the laboratory for crops of whole leafy vegetables (e.g. how to take a representative sub sample and to homogenize taking either outer or inner leaves or both). This makes the results of microbial contamination of whole leafy vegetables crops sampled at primary production or retail market more difficult to compare between different published studies and reports.

[17] EN/ISO 6579:2002. Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Salmonella* spp. International Organization for Standardization, Geneva, Switzerland.

[18] Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. OJ L 338, 22.12.2005, p. 1-26.

[19] EN/ISO 6579:2002. Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Salmonella* spp. International Organization for Standardization, Geneva, Switzerland.

[20] ISO 6887-4:2003. Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 4: Specific rules for the preparation of products other than milk and milk products, meat and meat products, and fish and fishery products. International Organization for Standardization, Geneva, Switzerland.

It is not possible to include prevalence data on contamination of leafy greens eaten raw as salads by *Salmonella* within Zoonoses monitoring data (according to the Directive 2003/99/EC[21]) since these data are aggregated into broad food categories, e.g. the single category of vegetables and fruits.

[21] Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC. OJ L 325, 12.12.2003, p. 31-40.

Table 1: Occurrence of *Salmonella* on whole leafy greens

Sampling place	Commodity	Country	Detection method	n	%	95% CI [(a)]	Sample size (g)	Reference
Farm	lettuce	Belgium	Either Vidas Easy SLM Assay (BioMérieux, France) or GeneDisc® PCR *Salmonella* (Pall, France) system for screening after 18h incubation at 37°C in buffered peptone water. In case of a positive signal, ISO 6579:2002[22] was used for isolation and confirmation.	88	0	[0,2.8]	25	(Holvoet et al., 2014b)
Farm	lettuce (romaine, batavia, trocadero, iceberg, maravella)	Spain	ISO 6579:2002	144	0	[0,1.7]	25	(Oliveira et al., 2010b)
Farm	lettuce	Norway	VIDAS SLM Assay (BioMérieux, France)	179	0	[0,1.4]	25	(Loncarevic et al., 2005)
Farm[23]	leafy greens (kale, spinach, amaranth, Swiss chard)	US	Modified BAM method	88	0	[0,2.8]	25	(Mukherjee et al., 2004)
	lettuce	US		55	2.0	[0.2,8.2]	25	(Mukherjee et al., 2004)
	cabbage	US		54	0	[0,4.5]	25	(Mukherjee et al., 2004)
Farm[24]	leafy vegetables (spinach, kale, collards, Swiss chards and 'mixed' (not further specified)	US	Modified BAM method	296	0	[0,0.8]	25	(Mukherjee et al., 2006)
	lettuce	US		157	0	[0,1.6]	25	(Mukherjee et al., 2006)
	cabbage	US		198	0	[0,1.3]	25	(Mukherjee et al., 2006)
Farmers' and public markets	lettuce	Canada	Health Canada Procedure MFLP-29 ''The Qualicon BAX System Method for the Detection of *Salmonella* in a Variety of Food and Environmental Samples''	128	0	[0,1.9]	25	(Bohaychuk et al., 2009)
	spinach	Canada		59	0	[0,4.2]	25	(Bohaychuk et al., 2009)

[22] EN/ISO 6579:2002. Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Salmonella* spp. International Organization for Standardization, Geneva, Switzerland.
[23] This study includes sampling at organic and conventional farms.
[24] This study includes sampling at organic, semiorganic and conventional farms.

Sampling place	Commodity	Country	Detection method	n	%	95% CI [(a)]	Sample size (g)	Reference
Retail distribution centres, farmers' markets and organic farms	head lettuce	Canada	Enrichment protocol described in the Health Canada Compendium of Analytical Methods MFHPB-20	155	0	[0,1.6]	25	(Arthur et al., 2007)
	leaf lettuce[25]	Canada		375	0.3	[0,1.2]	25	(Arthur et al., 2007)
Central produce supply station	spinach	Mexico	Modified ISO 6579:2002 method using LST broth as primary enrichment instead of buffered peptone water. for 18 to 24 h.	100	7.0	[3.2,13.3]	50	(Quiroz-Santiago et al., 2009)
	large lettuce	Mexico		100	7.0	[3.2,13.3]	50	(Quiroz-Santiago et al., 2009)
	romaine lettuce	Mexico		100	3.0	[0.9,7.8]	50	(Quiroz-Santiago et al., 2009)
	watercress	Mexico		100	7.0	[3.2,13.3]	50	Quiroz-Santiago et al., 2009)
	cabbage	Mexico		100	1.0	[0.1,4.6]	50	Quiroz-Santiago et al., 2009)
Entrance processing company	leafy greens (i.e. iceberg lettuce, endive, lollo rosso, curly endive, lollo bionda, green oak leaf lettuce, red oak leaf lettuce, baby leaf, red lettuce, radicchio rosso, rucola lettuce)[26]	The Netherlands	ISO 6579:2002	1860	0.38	[0.2,0.7]	25	(Pielaat et al., 2008)
Local retail	lettuce	UK	BS EN 12824:1998	151	0	[0,1.6]	25	(Little et al., 1999)
Retail	lettuce	UK	PHLS Standard Methods for Food Products F13	3198	0	[0,0.1]	25	(Sagoo et al., 2001)
Main distributors	lettuce	Norway	NMKL. no. 71	200	0	[0,1.2]	25	(Johannessen et al., 2002)
Retail	leafy greens[27] total	Spain	ISO 6579:2002	28	0	[0,8.5]	25	(Abadias et al., 2008)
	iceberg lettuce	Spain		5	0	[0,37.9]	25	(Abadias et al., 2008)
	lettuce hearts	Spain		3	0	[0,53.6]	25	(Abadias et al., 2008)
	oakleaf lettuce	Spain		5	0	[0,37.9]	25	(Abadias et al., 2008)
	trocadero lettuce	Spain		5	0	[0,37.9]	25	(Abadias et al., 2008)
	romaine lettuce	Spain		5	0	[0,37.9]	25	(Abadias et al., 2008)
	endive	Spain		5	0	[0,37.9]	25	(Abadias et al., 2008)

[25] Includes both conventional and organic leaf lettuce.
[26] The figures presented by this study also include the results of samples of other vegetables (red pepper and cucumber).
[27] Includes lettuce hearts, iceberg, oakleaf, trocadero, romaine and endive.

Sampling place	Commodity	Country	Detection method	n	%	95% CI [(a)]	Sample size (g)	Reference
Supermarkets and local markets	lettuce	Singapore	Modified ISO 6579:2002 method using Tetrathionate broth incubated at 42º C as a single selective enrichment medium.	13	0	[0,17.3]	25	(Seow et al., 2012)

(a): The credible interval was calculated using a Bayesian approach and taking as prior beta (1/2,1/2) (Miconnet et al., 2005).

Table 2: Occurrence of *Salmonella* on fresh-cut leafy greens

Sampling place	Commodity	Country	Detection method	n	%	95% CI [(a)]	Sample size (g)	Reference
Before and after processing (washing and packing)	savoy (curly leaves) and baby (flat leaves) spinach	US	MFLP-84. Health Canada. 'Isolation and Identification of *Salmonella* Species by Immunomagnetic separation (IMS)'	1311	0.4	[0.1,0.8]	25	(Ilic et al., 2008)
During processing	leafy greens (green Swiss chard, turnip greens, collards, cabbage, kale)	US	FDA BAM standard method	175	0	[0,1.4]	25	(Johnston et al., 2006)
End processing	fresh-cut leafy vegetables (i.e. radicchio, sugarloaf, curled endive, lettuce)[28]	Belgium	VIDAS Easy SLM Assay (BioMérieux, France) for screening after 18h incubation at 37°C in buffered peptone water. In case of a positive signal, ISO 6579:2002 was used for isolation and confirmation.	18	0	[0,12.9]	25	(Holvoet et al., 2012)
End processing	fresh-cut leafy vegetables[29]	The Netherlands	ISO 6579:2002	751	0	[0,0.3]	25	(Pielaat et al., 2008)
Main distributors	pre-cut salads	Norway	NMKL. no. 71	100	0	[0,2.5]	25	(Johannessen et al., 2002)
Retail	total	Spain	ISO 6579:2002	65	3.1	[0.6,9.5]	25	(Abadias et al., 2008)
	arugula	Spain		5	0	[0,37.9]	25	(Abadias et al., 2008)
	endive	Spain		21	0	[0,11.1]	25	(Abadias et al., 2008)
	lettuce	Spain		29	3.4	[0.4,15]	25	(Abadias et al., 2008)
	spinach	Spain		10	10.0	[1.1,38.1]	25	(Abadias et al., 2008)
Sampling at the production plant level	ready-to eat lettuce	Switzerland	ISO 6579:2002/amendedDAmd 1 (2006) using Modified semi-solid Rappaport Vassiliadis medium as a selective enrichment medium	142	0	[0,1.8]	10	(Althaus et al., 2012)
Supermarkets	total	Brazil	ISO 6579:2002	273	0.7	[0.2,2.3]	25	(Sant'Ana et al., 2011)
	collard greens	Brazil		24	0	[0,9.8]	25	(Sant'Ana et al., 2011)
	lettuce	Brazil		152	0.7	[0.1,3]	25	(Sant'Ana et al., 2011)

[28] The figures presented by this study also include the results of samples of other vegetables (parsley and chives).
[29] Also includes non-leafy greens.

Sampling place	Commodity	Country	Detection method	n	%	95% CI [(a)]	Sample size (g)	Reference
	arugula	Brazil		19	5.3	[0.6,22.1]	25	(Sant'Ana et al., 2011)
	watercress	Brazil		18	0	[0,12.9]	25	(Sant'Ana et al., 2011)
	chicory	Brazil		16	0	[0,14.3]	25	(Sant'Ana et al., 2011)
	escarole	Brazil		13	0	[0,17.3]	25	(Sant'Ana et al., 2011)
	cabbage	Brazil		11	0	[0,20]	25	(Sant'Ana et al., 2011)
	Swiss chard	Brazil		9	0	[0,23.8]	25	(Sant'Ana et al., 2011)
	spinach	Brazil		11	0	[0,20]	25	(Sant'Ana et al., 2011)
Supermarkets	total	Brazil	BAM standard official technique	111	3.6	[1.2,8.3]	25	(Froder et al., 2007)
	lettuce (iceberg, Boston and curly leaf lettuces)	Brazil		41	2.4	[0.3,10.8]	25	(Froder et al., 2007)
	mixed salads (mainly lettuce and other leaves)	Brazil		21	4.8	[0.5,20.2]	25	(Froder et al., 2007)
	watercress	Brazil		13	7.7	[0.8,30.7]	25	(Froder et al., 2007)
	spinach	Brazil		12	0	[0,18.5]	25	(Froder et al., 2007)
	chicory	Brazil		12	8.3	[0.9,32.8]	25	(Froder et al., 2007)
	arugula	Brazil		12	0	[0,18.5]	25	(Froder et al., 2007)
Supermarkets	total	Brazil	APHA Compendium of Methods 2001.	140	1.4	[0.3,4.5]	25	(Oliveira et al., 2011)
	lettuce	Brazil		26	0	[0,9.1]	25	(Oliveira et al., 2011)
	arugula	Brazil		6	0	[0,33]	25	(Oliveira et al., 2011)
	spinach	Brazil		9	0	[0,23.8]	25	(Oliveira et al., 2011)
	wild chicory	Brazil		13	0	[0,17.3]	25	(Oliveira et al., 2011)
	chicory	Brazil		11	18.2	[4,46.7]	25	(Oliveira et al., 2011)
	cabbage	Brazil		28	0	[0,8.5]	25	(Oliveira et al., 2011)
	Chinese cabbage	Brazil		13	0	[0,17.3]	25	(Oliveira et al., 2011)
	kale	Brazil		30	0	[0,8]	25	(Oliveira et al., 2011)
	watercress	Brazil		4	0	[0,44.5]	25	(Oliveira et al., 2011)

Sampling place	Commodity	Country	Detection method	n	%	95% CI [(a)]	Sample size (g)	Reference
Supermarkets and local markets	fresh-cut salads	Singapore	Modified ISO 6579:2002 method using Tetrathionate broth incubated at 42°C as a single selective enrichment medium	13	0	[0,17.3]	25	(Seow et al., 2012)

(a): The credible interval was calculated using a Bayesian approach and taking as prior beta (1/2,1/2) (Miconnet et al., 2005).

10. Analytical methods for the detection and enumeration of Norovirus in leafy greens

10.1. Standardisation of methods for detection and enumeration of Norovirus in leafy greens

Information on the standardisation of methods for detection of Norovirus in foods can be found in sections 4.3.2 of the Scientific Opinion of the EFSA BIOHAZ (EFSA Panel on Biological Hazards (BIOHAZ), 2011a).

In the absence of an efficient cell culture based detection system for human noroviruses, reverse transcription quantitative PCR (RT-qPCR) is the most widely used method to detect human noroviruses in foods including leafy greens. Standardised methods for the quantification and qualitative detection of Norovirus in food using real-time RT-qPCR have recently been published, namely ISO/TS 15216-1[30] and ISO/TS 15216-2[31]. These methods are technically complex, and their performance strictly according to their Technical Specifications can only be carried out in highly specialised and well-resourced laboratories. In particular, the production of the nucleic acid controls is challenging, and the availability of reliable quality control materials produced independently and EQA schemes will be necessary before there can be complete confidence in the concordance of results between laboratories.

The implementation of real-time RT-qPCR assays in food testing laboratories will be facilitated by commercially available separation and concentration systems or standardized ready-to-use real-time RT-qPCR kits (Stals et al., 2013). Such RT-qPCR kits as currently available do not completely conform to the ISO Technical Specifications, particularly in their use of differing amplification controls. Although the RT-qPCR assays are quantitative, the methods themselves may not allow consistent detection limits to be defined, due to the variable efficiencies of extraction of virus particles mediated by the multi-step sample treatment of the complex matrices. In some instances when the ISO Technical Specifications were used, the efficiency of recovery of spiked control virus was less than 1 % (Made et al., 2013). Furthermore, the assays use only $1/10^{th}$ or $1/20^{th}$ of the nucleic acid extract produced after sample treatment, and this, combined with the variable extraction efficiency can result in the method being unable to detect virus below e.g. 10^2 particles per sample. Further refinements of the existing methods are necessary to allow realistic detection levels to be consistently achieved. As Technical Specifications, the ISO methods can be revised at least every three years, and a future revision should consider the issues and challenges associated with methods as they currently stand.

Although reverse transcription quantitative PCR (RT-qPCR) is the most widely used method to detect human noroviruses, it (and other molecular-based methods) detects the presence of an RNA (or cDNA) fragment and is unable to differentiate between infectious and non-infectious viral particles. Thus, when using RT-qPCR for monitoring of food products for viral contamination the interpretation of the results is not straightforward (Stals et al., 2013), and there is difficulty in fully assessing the risk to human health. NoV RT-qPCR detection is unable to discriminate between infectious and non-infectious virus particles (Knight et al., 2013). Alternative strategies to overcome these drawbacks include amplification of the full length or multiple regions in the virus genome. However, while long-template real-time RT-qPCR may be possible in clinical samples (Kostela et al., 2008; Rodriguez et al., 2009), this technique may not be usable when detecting very low levels of foodborne viruses on food products as the decreased amplification efficiency substantially lowers the sensitivity of such PCR assays. Amplification of multiple viral genomic regions per foodborne virus tested is cumbersome and could be difficult to implement in routine analysis. As an alternative approach, several methods have been developed for analysis of the viral capsid integrity. Combining enzymatic treatments of RNA extracts with RNase and/or proteinase K with real-time RT- qPCR has been

[30] ISO/TS 15216-1: Microbiology of food and animal feed - Horizontal method for determination of hepatitis A virus and norovirus in food using real-time RT-PCR - Part 1: Method for quantification. International Organization for Standardization, Geneva, Switzerland.

[31] ISO/TS 15216-2: Microbiology of food and animal feed - Horizontal method for determination of hepatitis A virus and norovirus in food using real-time RT-PCR - Part 2: Method for qualitative detection. International Organization for Standardization, Geneva, Switzerland.

suggested. The use of integrated real-time RT-qPCR assays for detection of infectious virus particles have also been described: they are based on the assumption that infectious particles would more efficiently bind, for example with host cells (Li et al., 2011), or with porcine gastric mucin, than non-infectious particles (Tian et al., 2012). Although promising results for these alternative RT-qPCR methods have been published, further method development and testing on actual food products (including leafy greens) is needed. The application of these methods as an adjunct to the ISO Technical Specifications is discussed elsewhere by Knight et al. (2013).

11. Data on occurrence of Norovirus on leafy greens

As there is no routine or regular monitoring of leafy greens for the presence of Norovirus in most of the EU Member States, there is limited information on the general occurrence (prevalence) of noroviruses on leafy greens. There have been few research surveys conducted, and it is moreover difficult to harmonise the data from reported studies due to the nature and sensitivities of the detection methodology employed (Baert et al., 2011). Nevertheless, when studies have been performed, Norovirus genomic copies have been detected in samples of leafy greens.

Table 3 shows a summary of the occurrence of Norovirus on leafy greens available from the literature.

Table 3: Occurrence of Norovirus on leafy greens

Sampling place	Commodity	Sampling country	Number of samples analysed	Numbers of samples where Norovirus detected[(a)]	Numbers in positive samples	Reference
Catering company	leafy greens	Belgium	6	2	1.9 to 3.1 log genome equivalents g^{-1}	(Baert et al., 2011)
Supermarket	leafy greens	Canada	641	133 (NoV ggI)	1.4 to 8.3 log genome equivalents g^{-1}	(Baert et al., 2011; Mattison et al., 2010)
Supermarket	leafy greens	Canada	641	106 (NoV ggII)	1 to 6.4 log genome equivalents g^{-1}	(Baert et al., 2011; Mattison et al., 2010)
Food companies	leafy greens	France	6	2 (NoV ggI)	3.0 to 3.5 log genome equivalents g^{-1}	(Baert et al., 2011)
Food companies	leafy greens	France	6	1 (NoV ggII)	2 log genome equivalents g^{-1}	(Baert et al., 2011)
Point of sale	lettuce	3 European countries	149	2 (NoV ggI)	5 PCR-detectable units[32] per 25 g	(Kokkinos et al., 2012)
Point of sale	lettuce	3 European countries	126	1 (NoV ggII)	10 PCR-detectable units[32] per 25 g	(Kokkinos et al., 2012)

(a): ggI: genogroup 1; ggII: genogroup 2

[32] In this study, instead of using a calibrated quantitative assay a most probable number approach was used using end-point detection of RTPCR signal in dilutions of nucleic acid extracted from the sample, and therefore the data are expressed as 'PCR-detectable units'.

Information on the occurrence of Norovirus on leafy greens can be found in section 4.4.2 of the Scientific Opinion of the EFSA BIOHAZ (EFSA Panel on Biological Hazards (BIOHAZ), 2011a). In the study conducted in Canada, Belgium and France (Baert et al., 2011), Norovirus genomes were frequently detected in leafy greens. However, sequence confirmation was not successful for the majority of the samples tested. New European data can be found in the study of Kokkinos et al. (2012) in lettuce sold at retail in three European countries, where 2/149 and 1/126 samples were positive for Norovirus GI and GII respectively. In addition, adenovirus and Norovirus contamination was found in 1 sample of spinach sampled on-farm in South Korea (Cheong et al., 2009) and in 1 sample of spinach at point-of-sale in Istanbul, Turkey (Yilmaz et al, 2011).

Infection was rarely or not known to be related to the Norovirus-positive samples identified in the above studies. Consequently, a potential risk for infection cannot be excluded but the actual risk from RT-PCR Norovirus-positive produce is still unknown, as the detected virus may not have (all) been infectious. There is a need to thoroughly evaluate the public health risk of Norovirus (genomic copies) contamination derived from pro-active screening studies in foods/environmental samples that are not associated with reported outbreaks or illness (Baert et al., 2011). However, Norovirus should not be expected to occur in leafy greens, and its presence whether infectious or not signifies a failure in good hygienic practices somewhere along the supply chain.

It is not possible to include prevalence data on contamination of leafy greens eaten raw as salads by Norovirus within Zoonoses monitoring data (according to the Directive 2003/99/EC) since these data are aggregated within broad food categories, e.g. the single category of vegetables and fruits.

12. Mitigation options to reduce the risk to humans posed by *Salmonella* or Norovirus contamination in leafy greens

12.1. General mitigation options

Appropriate implementation of food safety management systems including Good Agricultural Practices (GAP), Good Hygiene Practices (GHP) and Good Manufacturing Practices (GMP) should be the primary objective of operators producing leafy greens eaten raw as salads. These food safety management systems should be implemented along the farm to fork continuum and will be applicable to the control of a range of microbiological hazards (Van Boxstael et al., 2013)[33]. Although some intervention strategies or control measures can be defined to prevent, limit the spread or sometimes reduce the level of contamination, the main focus for food safety management of leafy greens should be on preventive measures. Codes of practice and guidelines should specify the use of appropriate good agricultural and hygiene practices at farm level. Food safety management based upon Good Manufacturing Practices (GMP) and HACCP principles should be the objective of processors, distributors, retailers and caterers involved in production of leafy greens eaten raw as salads (Gil et al., 2013b).

Regulation (EC) No 852/2004 applies to food business operators producing or harvesting plant products and requires them to take adequate measures, as appropriate: (a) to keep clean and, where necessary after cleaning, to disinfect, in an appropriate manner, facilities, equipment, containers, crates, vehicles and vessels; (b) to ensure, where necessary, hygienic production, transport and storage conditions for, and the cleanliness of, plant products; (c) to use potable water, or clean water, whenever necessary to prevent contamination; (d) to ensure that staff handling foodstuffs are in good health and undergo training on health risks; (e) to prevent animals and pests from causing contamination; (f) to store and handle wastes and hazardous substances so as to prevent contamination; (g) to take account of the results of any relevant analyses carried out on samples taken from plants or other samples that have importance to human health; and (h) to use plant protection products and biocides correctly, as required by the relevant legislation. Adequate provision is to be made, where necessary, for washing food. Every sink or other such facility dedicated for the washing

[33] Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. OJ L 139, 30.4.2004, p. 1-54.

of food is to have an adequate supply of hot and/or cold potable water consistent with the requirements of Chapter VII and be kept clean and, where necessary, disinfected. Chapter VII of this Regulation (EC No 852/2004) also states that: (a) there is to be an adequate supply of potable water, which is to be used whenever necessary to ensure that foodstuffs are not contaminated; (b) where non-potable water is used, for example for fire control, steam production, refrigeration and other similar purposes, it is to circulate in a separate duly identified system. Non-potable water is not to connect with, or allow reflux into, potable water systems; (c) recycled water used in processing or as an ingredient is not to present a risk of contamination. It is to be of the same standard as potable water, unless the competent authority is satisfied that the quality of the water cannot affect the wholesomeness of the foodstuff in its finished form.

Where practicable, a comprehensive food safety control plan that includes a written description of each of the hazards identified in assessing environmental hygiene and the steps that will be implemented to address each hazard should be prepared at primary production. The description should include, but is not limited to an evaluation of the production site, water and distribution system, manure use and composting procedures, personnel illness reporting policy, sanitation procedures, and training programs. Furthermore, the following are examples of the types of records that should be retained:

- Microbiological testing results and trend analyses;
- Water testing results;
- Employee training records;
- Pest control records;
- Cleaning and sanitation reports;
- Equipment monitoring and maintenance records;
- Inspection/audit records and
- Temperature records.

Detailed records should be kept that link each supplier of the product with the immediate subsequent recipient of the food throughout the supply chain. The information should include, if available, the packer name, address, and phone number, date packed, date released, type of food including brand name and specific variety, lot identification, and number of items. In fresh-cut, pre-cut or ready-to-eat salad operations, multiple ingredients from different sources may be combined in a single package. This practice can complicate efforts to trace leafy vegetables to their source. The processors should establish and maintain records to identify the source of each ingredient in the product. The use of a radio frequency identification device (RFID) permits the tracking of leafy greens from the field to the retail level. Information can be continuously transmitted to the RFID tags, which can be interrogated remotely. The RFID system can be monitored via the Internet, and the technology can be used by both large and small operations (Gil et al., 2013b).

12.1.1. Environment

Primary production should not be carried out in areas where the known or presumptive presence of pathogens would lead to an unacceptable likelihood of transfer to horticultural crops intended for human consumption without a validated process kill step (CAC, 1969, 2003). This preventive measure is not always easy to implement as farmers may not control adjacent land activities or the land history does not include knowledge of the level of pathogens in the soil or time to reduce these to acceptable levels (Gil et al., 2013b; James, 2006; Suslow et al., 2003).

If the production site for growing leafy greens eaten raw as salads is located in a potentially hazardous location, intervention strategies focused on the construction of ditches and establishment of buffer areas will help to minimize transfer of microbial hazards (Abu-Ashour and Lee, 2000). It is also important to select management practices suitable for both the crop and the growing environment, including site management and crop rotation (Leifert et al., 2008). Preventive measures such as avoiding access of farm and wild animals to the site and to water sources should be developed and monitored for integrity, particularly near the time of harvest (CAC, 1969, 2003; CCFRA, 2002). Removing animal attractants and harborages in the production environment can impact on animal activity (Thorn et al., 2011). Physical barriers such as mounds, diversion berms, vegetative buffers, and ditches to re-direct or reduce runoff from animal production or waste management operations are sometimes required or introduced as prudent measures (James, 2006). Windbreaks and hedgerows may reduce dust or aerosol drift and attract other wildlife but may equally represent habitats for animal pests and should be selected and managed accordingly (Lowell et al., 2010). Distress machines and other repellent equipment, such as those emitting noise or calls (predator calls, sonic fences and ultrasonic rodent repellents) can reduce animal activity (Caro, 2005). Growers can use scarecrows, reflective strips or gunshots to ward off birds and pests from crops and also, mechanical traps (Gil et al., 2013b). Fields should be monitored for animal activity (e.g. presence of tracks, faeces and damage from grazing) particularly near harvesting. Plant debris and cull piles should be removed promptly from inside the production areas. There should be no plant refuse around the outside of the production areas or nearby to attract or harbour pests.

Production areas should be evaluated for hazards that may compromise hygiene and food safety, particularly to identify potential sources of faecal contamination. If the evaluation concludes that contamination in a specific area is at levels that may compromise the safety of crops, in the event of heavy rainfall and flooding for example, intervention strategies should be applied to restrict growers from using this land for primary production until the hazards have been addressed. Among the potential interventions, both water treatment and efficient drainage systems that take up excess overflows are needed to prevent the additional dissemination of contaminated water (FAO, 2003).

12.1.2. Manure, sewage and sludge

Appropriate storage and management of manure, including aerobic composting, anaerobic digestion, aeration of sludge, and stabilization is recommended to reduce residual pathogen population (Erickson et al., 2010; Suslow et al., 2003). Some of the treatment procedures to reduce or eliminate pathogens from contaminated manure are, for example: thorough composting, pasteurization, heat, drying, solar radiation, alkali digestion, sand drying or a combination of these (CAC, 1969, 2003; FDA, 2008). Proper composting of animal manure via thermal treatment has been described as an effective preventive measure (CFA, 2007; Erickson et al., 2010; Gil et al., 2013b; USDA, 2008). The pathogen-reduction criteria includes a temperature of at least 55ºC for 3 consecutive days in an aerated pile or 55ºC for 2 weeks in the hot zones of a windrow pile with 5 turnings (James, 2006). Soil amendment application techniques must control, reduce or eliminate the likely contamination of surface water and/or edible crops where these are being grown (EFSA, 2005; FAO, 2003; WGA, 2012). Close proximity to on-farm stacking of manure should be avoided. If the potential for contamination from the adjacent land is identified, intervention strategies (e.g. care during application and run-off controls) should be implemented to reduce the risk of contamination. Control of run-off or leaching by securing areas where manure is stored should be carried out. The proximity of wind-dispersed or aerosolized sources of contamination should be also minimized (Gil et al., 2013b). Direct or indirect contact between manure and fresh leafy greens should always be minimized while the time interval between the soil amendment application and time to harvest should be maximized. Pre-harvest intervals of 120 days are generally accepted in Good Agricultural Practices (GAP) guidance although 60 days is considered the minimum duration (Erickson et al., 2010). However, as discussed in section 3.2, survival of *Salmonella* depends on the type of organic wastes. For instance it seems longer in soil amended with fresh poultry manure (Islam et al. 2004a, Nyberg et al. 2010). In some Member States, a year between fresh slurries application and installation of leafy green production may be required. The Regulation (EU) No 142/2011 lays down some standards for composted manure and processed

manure placed on the market. Manure produced and used in the same farm may be applied to land without processing, if competent authorities do not consider it to present a risk for transmission of any serious transmissible disease. Competent authorities may also authorise the dispatch of unprocessed manure from other Member States.

A total of 10,135,745 tDS (tonnes of dry solids) of sewage sludge is produced in the EU, and about 40% of this is spread on all agricultural land (http://europa.eu.int/comm/environment/waste/sludge/index.htm) as a total or partial substitute for mineral fertilisers and to improve the soils by increasing their organic matter content. The sewage sludge directive (86/278/EEC[34]) prohibits the use of sludge on 'soil in which fruit and vegetable crops are growing, with the exception of fruit trees' and 'ground intended for the cultivation of fruit and vegetable crops which are normally in direct contact with the soil and normally eaten raw, for a period of 10 months preceding the harvest of the crops and during the harvest itself'. However practices on the use of sewage sludge may vary between Member States and these are outlined in various national guidelines. For example, in the UK the 'Safe Sludge Matrix' commonly referred to as the ADAS Matrix forms the basis of the agreement and consists of a table of crop types, together with clear guidance on the minimum acceptable level of treatment for any sewage sludge (often referred to as biosolids) based product which may be applied to that crop or rotation (available from http://adlib.everysite.co.uk/resources/000/094/727/SSMatrix.pdf). In the UK, since 1999, all untreated sludges have been banned from application to food crops. Stringent requirements apply where treated sludge is applied to land growing vegetable crops and in particular those crops that may be eaten raw (e.g. salad crops). Where the crop is a salad which might be eaten raw, the harvest interval must be at least 30 months. Where enhanced treated sludges are used, a 10 month harvest interval applies to both vegetables and salads. The same considerations apply to soil based greenhouses or polythene tunnel production.

12.1.3. Water

12.1.3.1. Water in primary production

The selection of appropriate irrigation sources as a preventive measure is very important, avoiding if possible, uncontrolled sources of water such as rivers and lakes (Gil et al., 2013b). For surface water, and even ground water interventions to reduce contamination from animals, as well as control of run-off are indispensable (Charatan, 1999; Gerba, 2009; Jones and Shortt, 2010; Oron et al., 2001; Pachepsky et al., 2011; Suslow et al., 2003). For instance this may mean setting distance limits from the water resources used for irrigation, for the animal raising building, the stored effluents, or the lands spread with manure. Additional protection of water sources from seepage is needed where water supplies are delivered in peri-urban or mixed agricultural areas. Mitigation strategies are likely to vary depending on the level of risk. As outlined in Section 3.3, for both *Salmonella* and Norovirus, processes which wet the edible portions of the crop represent the highest risk and these include spraying prior to harvest, direct application of fertilizers, pesticides and other agricultural chemicals and overhead irrigation. Sub-surface or drip irrigation which results in no wetting of the edible portions are of lower risk.

To reduce microbial contamination from irrigation water, growers should establish a risk assessment system for evaluating the potential impacts of environmental factors on the microbial quality of irrigation water and the implementation of control and monitoring systems. Sanitary surveys of canals and ditches should focus on the integrity of surrounding bank systems focusing on potential point source and non-point source confluences (e.g. drainage into these systems) (Jones and Shortt, 2010). Since *E. coli* is an indicator microorganism for faecal contamination in irrigation water, growers should arrange for periodic testing to be carried out to inform preventive measures. *E. coli* is also suggested as an indicator microorganism for faecal contamination in irrigation water, which should be periodically tested.

[34] Council Directive 86/278/EEC of 12 June 1986 on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture. OJ L 181, 4.7.1986, p. 6-12.

The frequency of testing will vary depending on the water source and the risks of environmental contamination including intermittent or temporary contamination (e.g. heavy rain, flooding, etc.) (Gerba, 2009; Jones and Shortt, 2010). In most instances, pathogen contamination of such waters is a 'rare' event, so direct pathogen screening is likely to be ineffective. In addition the detection of pathogens is expensive, time consuming, and complex due to pathogen variability (Savichtcheva and Okabe, 2006). Consequently, pathogens are not routinely monitored in processing areas or in well or borehole waters. Instead indicator organisms are routinely used by environmental agencies and public health organizations to verify effective implementation of good agricultural practices (Efstratiou et al., 2009; Ferguson et al., 2012; Wilkes et al., 2009). The indicators typically consist of coliform bacteria, Enterococci or *Escherichia coli*[35] (Suslow et al., 2003). The presence/numbers of coliforms is assumed to indicate unhygienic working conditions or failures in inactivation treatments, faecal pollution and an association with ecologically similar enteric pathogens (Holvoet et al., 2014b).

Microbial indicators of faecal contamination do not necessarily reflect the input of enteric pathogens, however, in some waters predictive values have been reported especially between faecal indicators and pathogens (Harwood et al., 2005; Hegarty et al., 1999; Lemarchand and Lebaron, 2003; Lipp et al., 2001; Schets et al., 2005; Wilkes et al., 2009). Variations in pathogen input (i.e., prevalence in population), dilution, retention, and die-off result in conditions where relationships/correlations between the presence or levels of a pathogen and an indicator are random, site-specific, or time-specific events (Payment and Locas, 2011). As a result, there is clearly no particular indicator that is suitable for all pathogens in all environments (Harwood et al., 2005; Payment and Locas, 2011; Wilkes et al., 2009; Yates, 2007). However, there is a greater likelihood of detecting pathogens when the level of indicator microorganisms is high (Savichtcheva and Okabe, 2006). The presence of bacterial pathogens and indicator bacteria can show a seasonal effect especially in water (Naumova et al., 2007; Wilkes et al., 2009). Both tend to be more often present during the months with higher temperatures (Holvoet et al., 2014b).

The microbiological data obtained from applying the sampling plan will serve as an input for the microbial risk assessment of the environmental contamination including intermittent or temporary contamination. The frequency of testing may vary depending on the water source and the risks of environmental contamination including intermittent or temporary contamination events. These recommendations can be summarized as follows:

(a) Make an inventory of the sources of pathogenic microorganisms likely to contaminate the irrigation water.

(b) Examine the levels of indicator microorganisms present at different times of the year, according to seasonal variations in irrigation water from different sources.

(c) Establish a sampling programme for the irrigation water based on the examination of current and historical data and with a number of samples, a geographical distribution of the sampling points and a sampling frequency to ensure that the test results are as representative as possible.

(d) If necessary, disinfect the irrigation water maintaining residual disinfectant concentrations within a locally predetermined range and minimizing the transit time.

None of these parameters can predict the presence of pathogens, however, temperature and *E. coli* concentration provide some information concerning the most critical periods for possible pathogen contamination of the produce and water, especially open water reservoirs or surface waters which might be used for irrigation(Schilling et al., 2009). When critical periods are identified it must induce a higher state of awareness to prevent contamination of the produce by e.g. contaminated irrigation

[35] Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. OJ L 338, 22.12.2005, p. 1-26.

water. Alternatively, faecal indicator bacterial tests can be used to monitor water quality, but these do not always correlate well with the presence of pathogens (Holvoet et al., 2014b).

Treating water during storage, between storage and delivery systems and while in the delivery systems as well as maintaining disinfectant residual concentrations within a locally predetermined range and minimizing the transit time represents another class of mitigation strategy. Water treatments include coagulation, flocculation, filtration, and disinfection (Gil et al., 2013b). Solar radiation is also suggested as a contributor to reducing the levels of pathogenic microorganisms (Caslake et al., 2004). Other intervention strategies have been considered to improve microbial quality of surface wastewaters, such as sand filtration or storage in catchments or reservoirs to achieve partial biological treatment before use (Carr, 2004). Special attention to the water quality should be considered when using delivery techniques (e.g. sprayers) that expose the edible portion of leafy greens directly to water, especially close to harvest time (CAC, 1969, 2003; Marites et al., 2010; Suslow, 2010). The control of water quality in intermittent supplies represents a significant challenge, because the risk of backflow increases significantly due to reduced pressure (Gil et al., 2013b). Preventive measures to maintain microbial quality include maintaining disinfectant residual concentrations within a locally predetermined range and minimizing the transit time (WHO, 2004). Disinfectant treatments of surface or well water include chlorination, pH shock, peroxyacetic acid, hydrogen peroxide, electrochemical disinfection and UV treatment. Ozonation and chlorine dioxide injection have also been described as possible disinfection treatments for irrigation water (Suslow, 2004, 2010).

Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs defines potable water as 'meeting the minimum requirements laid down in Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption'. Furthermore, clean seawater is defined as 'natural, artificial or purified seawater or brackish water that does not contain microorganisms, harmful substances or toxic marine plankton in quantities capable of directly or indirectly affecting the health quality of food; 'clean water' means clean seawater and fresh water of a similar quality'. Therefore the use of clean water is permitted for primary production where it is used in primary washing steps for raw product. However there may also be requirements for the use of potable water in primary production which will be similar to that for a food business operator.

12.1.3.2. Process wash water

Mitigation strategies aiming to reduce risks of microbial contamination include treatment and quality maintenance of process wash water to reduce the build-up of microorganisms (FDA, 2008). The water treatment process should be monitored and controlled. Control of the sanitary quality of water is technologically feasible but requires strict management of operating practices (Lopez-Galvez et al., 2010; Luo et al., 2011; Suslow, 1997). Some companies use chlorine or other disinfection agents to control microbial load in the process wash water (Gil et al., 2009). Chlorine in the form of sodium hypochlorite granules, tablets or liquid is the most commonly used disinfection agent (Suslow, 2001). The use of other disinfection techniques such as electrolyzed water, UV-C light, ozone, hydrogen peroxide, peroxyacetic acid, etc have also been recommended (CAC, 2003; FAO, 2003; FDA, 2009; Suslow, 2004; WGA, 2012). The levels of disinfection agents should be monitored and controlled to ensure that they are maintained at effective minimum concentrations (Lopez-Galvez et al., 2009). In fresh-cut processing plants, microbial and physico-chemical quality of process wash water decreases rapidly due to the continuous addition of organic matter to the washing tanks. To maintain the quality of the process wash water the use of a residual concentration of a disinfection agent in the wash water is critical for preventing pathogen survival and transfer. Maintaining a relatively consistent level of a disinfection agent during commercial fresh-cut wash operations is a technical challenge in practice because of the rapid reaction of the disinfectants with organic materials in the produce wash solution (Luo et al., 2011). Recent studies highlight that maintaining a residual concentration of 1 mg/litre free chlorine in the process wash water, kept bacterial contamination below 2.7, 2.5, and 2.5 log CFU/100 ml for tap water and artificial process water with COD values of 500 and 1,000 mg O_2/litre, respectively (Van Haute et al., 2013). However, residual concentrations between 3 and 5 mg/litre

completely inhibited microbial contamination in artificial process water with COD values of 500 mg O_2/litre (Gil et al., 2013a).

12.1.4. Equipment

The Codex Code of Hygiene Practice for Fresh Fruits and Vegetables establishes sanitary practices that might be considered as preventive measures to avoid contamination of equipment associated with growing and harvesting (CAC, 1969, 2003). Interventions to reduce or eliminate contamination through equipment associated with growing and harvesting include the identification of specific hygiene and maintenance requirements for each piece of equipment that is used and the type of fruit or vegetable associated with it (FDA, 2008; Marriott, 1989). Intervention strategies should be managed to discard equipment and tools that can no longer be kept in a hygienic condition (Gil et al., 2013b). Cleaning of contaminated containers to control, reduce or eliminate microbial risks should be a regular and consistent operational practice. Identification and segregation is an intervention strategy to avoid the use of contaminated equipment during harvesting (Giese, 1991).

When sampling plans and methodology are properly designed and performed, microbiological testing using a process monitoring approach (as used for animal feed production (EFSA, 2008) can be a useful tool to evaluate and verify the effectiveness of safety and sanitation practices, provide information about an environment, a process, and even a specific product lot. The intended use of information obtained (e.g. evaluating the effectiveness of a sanitation practice, evaluating the risk posed by a particular hazard, etc.) can aid in determining which microorganisms are most appropriate to test for. Test methods should be selected that are validated for the intended use. Consideration should be given to ensure proper design of a microbiological testing program. Trend analysis of testing data should be undertaken to evaluate the effectiveness of food safety control systems.

12.1.5. Workers

It is recommended to have standard enforceable policies and provide training in sanitation to all employees working in primary production[36]. To support this training, hygiene and sanitation facilities are recommended to ensure that an appropriate degree of personal hygiene can be maintained (CAC, 1969, 2003; WGA, 2012). If human activity is the reason for contamination, interventions aimed at controlling microbial risk will be necessary. People known, or suspected, to be suffering from, or to be a carrier of a disease or illness likely to be transmitted through fresh leafy vegetables should not be allowed to enter any food handling area (FAO, 2003). If a worker has a potential source of contamination such as cuts or wounds, these should be covered by suitable waterproof dressings before permitted to continue working (Ritenour et al., 2010; WGA, 2012).

Each businesses operating primary production should have written standard operating procedures (SOPs) that relate to health, hygiene and sanitary facilities. The SOPs should address worker training, facilities and supplies to enable workers to practice proper hygiene, and company policies relating to expectations for worker hygiene as well as illness reporting. All workers should wash their hands properly using soap and potable, running water before handling leafy vegetables, particularly during harvesting and post harvest handling. Workers should be trained in proper techniques for hand washing and drying and should wash hands before entering production areas. Separate sinks for hand washing must be provided for staff at all stages of the leafy green food chain and these must have taps designed to prevent the spread of contamination. If gloves are used, a procedure for glove use in the field should be documented and followed. If the gloves are reusable, they should be made of materials that are readily cleaned, sanitized, and stored appropriately. If disposable gloves are used, they should be discarded when they become torn, soiled, or otherwise contaminated. Non-essential persons and casual visitors, particularly children, should not be allowed in the harvest area as they may present an increased risk of contamination.

[36] Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. OJ L 139, 30.4.2004, p. 1-54.

Areas away from the field and packing lines should be provided for workers to take breaks and eat. These areas should contain toilets and hand washing facilities so workers can practice proper hygiene. All workers should be trained in proper use of hygienic facilities. Training should include toilet use, proper disposal of toilet paper or equivalent, and proper hand washing and drying procedures. As far as possible, such facilities should be located close to the field and readily accessible to the work area. Sanitary facilities should be located in a manner to encourage their use and reduce the likelihood that workers will relieve themselves in the field. Facilities should be in sufficient number to accommodate personnel (e.g. 1 per 10 people) and be appropriate for both genders if workforce contains both males and females. Portable facilities should not be located or cleaned in cultivation areas or near irrigation water sources or conveyance systems. Growers should have a standard plan that identifies the areas where it is safe to put portable facilities and to prevent traffic in case of a spill. Facilities should include clean running water, soap, toilet paper or equivalent, and single use paper towels or equivalent.

Microbial contaminants on hands can comprise 1) resident microflora (of no pathogenic significance in this context) and 2) transient contaminants (of possible significance). Transient contaminants will be acquired by touch, are superficially located and very readily lost. This means there will probably be only a short duration between deposition on the hands and transfer to a food product. Given these dynamics, sampling hands provides no verification that hand transfer of pathogens has not or will not take place. This is the case whether pathogens or surrogates, such as faecal microflora, are looked for. Verification of control in this area is by the process (hand hygiene) not the product (hands). In healthcare, this is achieved by training and observational audit. Similar approaches are advocated for hand hygiene in hospitals and other health-care establishments (see WHO hand hygiene guidance: http://whqlibdoc.who.int/publications/2009/9789241597906_eng.pdf).

12.1.6. Final product

Harvested leafy greens are not subjected to physical interventions that completely eliminate microbial contamination. Some primary producers base their control intervention strategies on testing for microbial contamination. Pathogen contamination at primary production is usually at low prevalence and has been reported to be less than 0.5% of all tested lots of leafy greens (D'Lima and Suslow, 2009; ICMSF, 2002; Pielaat et al., 2008). Previous studies have shown that pathogen distribution in a field can be heterogeneous and detecting the most heavily contaminated fields can be difficult even when using a statistically valid sampling design (Gil et al., 2013b; Gutierrez-Rodriguez et al., 2012; ICMSF, 2002).

Technologies currently available for use by the leafy greens industry fall short of being able to guarantee an absence of *Salmonella* or Norovirus on leafy greens at primary production. Washing procedures for minimally processed products are undertaken to eliminate general field dirt and debris and cooling and cleaning of the leafy greens. However where contamination has occurred, even with adequately operated and monitored washing procedures, at best, a reduction of 1 up to 2 log units in microbial contamination is achieved (FAO, 2003; Lopez-Galvez et al., 2010). It is therefore essential to prevent the build-up of the microbial load in the water during the washing procedure which as a consequence may lead to transfer and dispersion of microorganisms from an initial localised contamination on input to the washing step to a great number of fresh-cut leafy greens at the end the washing process.

There have been many attempts to develop effective chemical or physical decontamination interventions for leafy vegetables and/or improve the performance of current interventions such as cold plasma and irradiation as non-thermal antimicrobial treatments. Physical intervention strategies for pathogen inactivation on produce include ionising irradiation (Farkas, 2006; Fonseca, 2006), high pressure processing (Arroyo et al., 1997), high-intensity electric field pulses (Mosqueda-Melgar et al., 2008), and ultraviolet irradiation treatments (Allende, 2006). Application of these techniques may be limited by their impact on the quality of leafy greens, by the accessibility of the leaf surface (e.g. to ultraviolet irradiation) and a lack of application of these decontamination interventions other than in

experimental settings. Ionising radiation has been shown to greatly reduce microbiological contamination without damaging the texture/colour of produce (Niemira et al., 2003). This technique is currently not permitted in the EU for this type of product.

There are also likely to be effects of interference of pathogens by the indigenous competing flora. However these strategies are either only available in experimental settings or are unable to provide an effective intervention to eliminate contamination by microbiological pathogens.

In retail and catering environments, adequate segregation and hygiene is important to prevent cross-contamination from other produce or other foods as well as contamination from food handlers. Open food counters such as salad bars present additional problems and these should have a high level of supervision, adequate utensils for service and may be best positioned next to a busy service counters such as a delicatessen counter. Products should be removed from sale if there are long periods when they may be unsupervised and proper storage and control should be carried out to minimize proliferation of microbiological contamination.

12.1.7. Training and education of workers

All persons involved in the handling of leafy greens should receive hygiene training appropriate to their tasks and should be periodically assessed while performing their duties to ensure tasks are being completed with due regard to good hygiene and hygienic practices. Training should be delivered in a language and manner to facilitate understanding of the information and expectations. Training programs should be designed to help personnel understand what is expected of them and why and the importance of using hygienic practices should be emphasised. The following training considerations should be addressed:

- Longstanding entrenched behaviours, attitudes and social taboos
- Transient nature of workforce with no prior training in food safety and hygiene
- Children/infants, who may accompany parents working in the field with the potential for transfer of those pathogens with a human reservoir
- Diverse cultural, social and traditional practices
- Literacy and education level
- Language and dialect of trainees
- Need to make food safety practices realistic and easy to implement (identify enabling factors, motivators and incentives)
- Raising awareness among trainees of symptoms and signs of disease and encourage them to act upon it (take personal responsibility for health)
- Importance of food safety training when new crops are being grown for the first time.
- Training programs should be regular, updated particularly when there is a change in product variety or process recorded, monitored for effectiveness and modified when necessary.
- Training on hand hygiene is particularly important.

For these involved with all the stages after harvest (including those involved with logistics), training in management of the cold chain should be given where appropriate.

12.1.8. Consumers

Clear information (including labelling) should be provided to consumers on appropriate handling of leafy greens which includes specific directions for product storage, preparation, intended use, 'use-by' date or other shelf-life indicators. Consumer information on handling leafy greens eaten raw as salads should cover:

- Selection of produce at retail and prevention of mechanical damage which may minimize internalization and proliferation of microbiological contamination;
- Transporting time to home to be kept as short as possible;
- Appropriate temperature control during storage;
- Washing leafy greens when appropriate with potable water;
- Correct hand washing methods using soap and potable water before and after handling;
- Appropriate handling to avoid cross-contamination with pathogens from various sources e.g., raw meats, hands, sinks, cutting boards etc.

Consumers should be provided with clear guidance on how to safely handle of leafy greens eaten raw as salads. This should include clear and easy-to-read labelling of bagged salads including those products where there is advice on those that require further washing before consumption and those that do not. A recent expert group (Palumbo et al., 2007) concluded that leafy green salad in sealed bags labelled 'washed' or 'ready-to-eat' do not need additional washing prior to consumption unless specifically directed on the label. This group concluded that additional washing is not likely to enhance safety and may introduce cross-contamination risks during washing from food handlers or food contact surfaces.

12.2. Specific mitigation options to reduce the risk of *Salmonella* contamination

As *Salmonella* has reservoirs in domestic as well as wild animals, birds and humans, the main mitigation options for reducing the risk of contamination of leafy greens are to prevent direct contact with faeces as well as indirect contact through slurries, sewage, sewage sludge, contaminated soil, water, equipment or food contact surfaces. Compliance with hygiene requirements, in particular hand hygiene, is an absolute necessity for all food handlers.

At primary production, assessment of risks for *Salmonella* contamination from the environment should aim to reduce risks from previous cultivation or adjacent land use (particularly when associated with domestic animal production) as well as attractants and harbourage of wild animals and pests. Particular attention should be paid to appropriate treatment, storage and application of both manure and sewage sludge if used since this bacterium survives in water, including the possibility of contaminating water used for irrigation. Care should also be taken to prevent the use of equipment contaminated with *Salmonella*, particularly segregation from equipment that has come into contact with animals. Persons handling food during harvesting, processing (as well as during subsequent processing) are a potential source of *Salmonella* contamination, and adequate toilet and hand-washing facilities must be provided at production areas together with the exclusion of persons with symptoms of gastroenteritis. Compliance with hygiene requirements, in particular hand hygiene, such as effective washing is an absolute necessity for all food supply chain employees, and should be emphasised in local codes of practice and training manuals.

During processing, cooling and washing all necessary steps to prevent contamination by *Salmonella* should be carried out, however these processes at best are aimed at preventing contamination or outgrowth. Where contamination has occurred at primary production, even with adequately operated and monitored washing procedures, at best, reduction of microbial load with usually no more than 1

and up to a maximum of 2 log unit reduction in pathogen contamination can be achieved in the final product.

During distribution, retail, catering and handling in domestic environments, all reasonable steps should be taken to prevent cross-contamination of *Salmonella* from other foods, as well as from food handlers.

12.3. Specific mitigation options to reduce the risk of Norovirus contamination

Information on existing preventive measures for Norovirus contamination in place according to current EU legislation and control options for leafy greens can be found in sections 6.2 of the Scientific Opinion of the EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards (BIOHAZ), 2011a), in the Codex Committee on Food Hygiene guidelines for control of virus contamination of food (CAC, 2012), and in guidance sheets produced by the FP7 project 'Integrated monitoring and control of foodborne viruses in European food supply chains' (available at http://www.eurovital.org/). No specific EC legislation exists for viruses in leafy greens.

12.3.1. Sewage and sludge

Since humans are the reservoir of Norovirus pathogenic to humans the main sources within the environment for contamination of food include sewage, sewage sludge and human faecal contaminated water where the virus can be found at high concentrations (Rao et al., 1986). The process of sewage treatment produces high volumes of sludge; the Urban Waste Water Treatment Directive 91/271/EEC[37] encourages the application of sewage on to agricultural land as fertiliser; however to reduce the likelihood of pathogen contamination of crops subsequently grown, the Directive forbids the application to soil on which vegetable crops are grown less than 10 months prior to harvest. The reduction in infectivity of Norovirus in sewage-amended soil over this period is not known.

12.3.2. Water

The Codex Committee on Food Hygiene guidelines for control of virus contamination of food (CAC, 2012) recommend that potential sources of viral contamination of the environment should be identified prior to production activities, and that primary food production should not be carried out in areas where the presence of viruses may lead to the viral contamination of food, e.g. in close proximity to a sewage treatment plant where there might be discharges of sewage water in the surface water, as even sewage treated by systems such as filtration can contain high levels of Norovirus (Nenonen et al., 2008).

Norovirus may be found in supply waters used in primary production, e.g. ground water (Borchardt et al., 2012; Cheong et al., 2009) and river water (Maunula et al., 2012; Wyn-Jones et al., 2011) which they can contaminate via the ingress of sewage, e.g. through outflow from a sewage treatment plant, or failure of a sewage system. Norovirus GI and GII have been detected in irrigation water used in leafy green production (Kokkinos et al., 2012). Fresh water in the environment allows for the survival of enteric viruses (Rzeżutka and Cook, 2004), and it is highly likely that Norovirus will survive in an infectious state in river and groundwater from introduction via a sewage pollution event to application of the water to leafy greens during irrigation, washing or pesticide application (Verhaelen et al., 2013b). Untreated water used in primary production and/or processing is therefore a significant vehicle for virus contamination of leafy greens. The Codex Committee on Food Hygiene guidelines for control of virus contamination of food (CAC, 2012) recommend that efforts should be made to use only clean or potable water during production and processing. At production, an assessment should be performed of the microbial quality of the sources of water used, including an assessment of possible human faecal contamination sources of the water (sanitary survey). Corrective actions should be taken if sources of contamination are identified. Possible corrective actions include disinfection e.g. by chlorine. The effectiveness of chlorine against Norovirus is not fully defined due to the lack of an

[37] Council Directive of 21 May 1991 concerning urban waste water treatment. OJ L 135, 30.5.1991, p. 40-52.

infectivity assay, although studies observing the effect of chlorination on detectable viral RNA (Shin and Sobsey, 2008) indicate that chlorine concentrations used to treat drinking water are likely to be effective. The risk of virus contamination of leafy greens via contaminated water may also be reduced by using subsurface or drip irrigation rather than spray irrigation (Hamilton et al., 2006).

12.3.3. Equipment

Equipment such as knives used in harvesting or trimming, conveyor belts or utensils used for processing, may act as vehicles for cross-contamination of produce. For example, a study using murine Norovirus as a model demonstrated that knives and graters processing contaminated fresh produce items including cucumbers and tomatoes can become contaminated by the virus and contaminate subsequently processed items (Wang et al., 2013). Regulation EC No 852/2004 requires that equipment which comes into contact with food should be effectively cleaned and where necessary disinfected. The efficacy of currently available surface disinfection treatments against Norovirus is not fully understood, and EFSA has recommended that effort should be focussed on avoiding viral contamination (EFSA Panel on Biological Hazards (BIOHAZ), 2011a).

12.3.4. Workers

Persons handling food during harvesting, processing and catering are potential sources of Norovirus contamination of foods. Viruses can be transferred from the hands onto food items or food preparation surfaces, particularly under moist conditions (Bidawid et al., 2000). In a study of leafy green production sites in three European countries (Kokkinos et al., 2012) enteric viruses including Norovirus were detected in swabs from the harvesters' hands. It is stated (CAC, 2012; EFSA Panel on Biological Hazards (BIOHAZ), 2011a) that persons with symptoms of gastroenteritis should be excluded from working in food production until the symptoms have subsided, e.g. for 48 hours. However, as pre- and post-symptomatic shedding can occur (Atmar et al., 2008) this exclusion procedure may not entirely prevent the possibility of food contamination with Norovirus. Compliance with hygiene requirements, in particular hand hygiene, such as effective washing is an absolute necessity for all food supply chain employees, and should be emphasised in local codes of practice and training manuals.

12.3.5. Final product

Information on effects of treatments used in food processing on noroviruses can be found in sections 4.2. and 4.2.1. of the Scientific Opinion of the EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards (BIOHAZ), 2011a).

Many leafy greens are eaten without cooking, and therefore mitigation options are limited, e.g. heating the product sufficiently to inactivate viruses is not applicable. Disinfection of leafy greens is performed by some producers/processors, commonly on salad items to be sold bagged and ready-to-eat; however disinfection procedures used in the food industry may only have limited effect on enteric viruses (Seymour and Appleton, 2001).

Disinfection is likely to be ineffective if Norovirus are internalised within the tissues of leafy greens. Whether virus internalisation occurs naturally or frequently in actual crop production settings is unknown (see 3.1. 1).

12.3.6. Conclusion

Attention should be paid to the selection of the water source for irrigation, pesticide application and in particular avoiding the use or the ingress of sewage water. The requirements for growers and producers producing or harvesting leafy greens are very general in nature and leave room for interpretation i.e. use potable water, or clean water, whenever necessary to ensure that foodstuffs are not contaminated.

Apart from avoiding the use of sewage-contaminated water at all stages of the supply chain, the main mitigation options for reducing the risk of Norovirus contamination on leafy greens are adherence to hand hygiene by food handlers at all stages of the supply chain (see section 8.1.5 and 8.1.8).

Compliance with existing prerequisite programs such as Good Agricultural Practices (GAP) and Good Manufacturing Practices (GMP), and with recommended Codes of Practices and guidance such as the relevant Codex guidelines, will assist Norovirus risk mitigation strategies.

However the evaluation of water quality, water treatment technologies or other risk mitigation solutions (e.g. selection of appropriate agents for cleaning and disinfection) for Norovirus are hampered by the current lack of suitable methods for *in vitro* determination of Norovirus infectivity (Richards, 2012) and current NoV RT-qPCR detection and monitoring methods are unable to discriminate between infectious and non-infectious virus particles (Knight et al., 2013) (see section 12.1).

13. *E. coli* as a microbiological indicator in leafy greens

The detection of pathogens in leafy greens is expensive, time consuming, and complex (Savichtcheva and Okabe, 2006). Furthermore, human pathogenic bacteria in food, and in particular in plant production environments and field crops, are often heterogeneously distributed and present in low numbers making detection difficult. Many food processing sites also prefer not to isolate enteric pathogens in their on-site laboratory but rather they elect to have testing performed by an externally validated laboratory. Consequently, pathogens are most of the time not directly monitored in plant production areas, in surface or well waters or in food manufacturing sites. Instead indicator organisms are routinely used by the industry, environmental agencies and public health organizations to verify effective implementation of Good Agricultural Practices (GAP) and Good Manufacturing Practices (GMP) (Efstratiou et al., 2009; Ferguson et al., 2012; Wilkes et al., 2009). However it should be emphasised that testing should never be relied upon as a food safety management strategy, but rather should complement existing strategies (Good Agricultural Practices (GAP), Good Hygiene Practices (GHP) and Good Manufacturing Practices (GMP)).

Indicator organisms typically consist of coliform bacteria, enterococci or *Escherichia coli*[38] (Suslow et al., 2003). Their presence is assumed to indicate unhygienic working conditions, faecal pollution or failures in control measures. The term 'index' organisms has been introduced for marker organisms whose presence in numbers exceeding given numerical limits indicates the possible occurrence of ecologically similar pathogens. This is in contrast to the term 'indicator' organisms which is suggested for those marker organisms whose presence in given numbers points to 'inadequate processing' for safety. A positive test for indicator organisms does not necessarily point to the presence of pathogenic organisms in the same commodity. The detection of an index organism in a food, however, provides evidence that a related pathogen may also occur, if not in the tested consignment, then in a previous or later one. Index organisms may not be considered valid as surrogate markers for foodborne pathogens unless a correlation between their occurrence and that of well-defined pathogens has been established (Mossel et al., 1995).

Bacteria such as *E. coli* can have a dual purpose in the same food (e.g. leafy greens): *E. coli* can function both as an indicator organism to verify Good Agricultural Practices (GAP) and Good Manufacturing Practices (GMP) and absence of significant faecal contamination and to some extent also as an index organism. *E. coli* is an established faecal (human or animal) marker organism: its presence provides evidence of an increased likelihood of potential contamination of food or water by ecologically closely related pathogens (Mossel et al., 1995).

Nonetheless, *E. coli* has its limitations. To be an effective index organism, it must be as resistant or as persistent as the pathogen it is used for as a surrogate, and must share the same ecological niche. It is

[38] Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. OJ L 338, 22.12.2005, p. 1-26.

generally assumed that this is the case, but there is little evidence for a definitive correlation between the presence or levels of *E. coli* and the presence of pathogens, including *Salmonella* and enteric viruses (Busta et al., 2003). Wilkes et al. (2009) described seasonal relationships between indicator bacteria and pathogens for surface waters in Canada and concluded that *E. coli* numbers were the most useful classifiers of pathogen presence. When *Salmonell*a was detected (per litre) in the water sample the *E. coli* median value was 365 cfu/100 ml, whereas in the case of samples with no *Salmonella* detection, the *E. coli* median value was 54 cfu/100 ml. Overall rainfall and discharge were primarily positively associated with densities of indicator bacteria and pathogen detection. Another study, noted some limitations on the use of faecal indicator bacteria for the microbial assessment of roof harvest rain water quality in particular because of their poor correlation with pathogenic microorganisms (including *Salmonella, Campylobacter, E. coli* O157, other STEC, *Cryptosporidium, Giardia, Aeromonas hydrophilia, Legionella pneumophila*). In this study 12% of samples (n = 100) had <1 cfu *E. coli*/100 ml but were positive for one or more pathogens, but it should be highlighted that in the study pathogen detection was performed by PCR testing (Ahmed et al., 2010).

In a study at retail, *Salmonella* was found in some samples of leafy greens but there were insufficient contaminated samples to establish any relationship between the presence of the pathogen and numbers of *E. coli* present (Sagoo et al., 2003b).

Overall, as was mentioned above (section 10) there is little use (in particular for low prevalence of pathogens expected to be present in the case of leafy greens) in looking consistently for pathogens themselves in the end product or raw material or production environment because if no enteric pathogens are detected in a particular portion of a given food consignment, the result is at the very best of significance only to the specific consignment that has been sampled. Furthermore, the restricted number of samples tested is statistically insufficient to detect a low prevalence of contamination (< 1%) of pathogens. However, if the absence of a suitable marker organism (e.g. *E. coli* in leafy greens) can repeatedly be verified in a series of samples from a processing line (thus as a process criterion), then the probability that the commodity is contaminated with enteric pathogens is reduced (Mossel et al., 1995).

When analysing pre-cut ready-to-eat fruit and vegetables in the scope of the verification of compliance with the currently established processing hygiene microbiological criterion for *E. coli*, EN/ISO standard methods 16649-1[39] or 16649-2[40] are prescribed in Regulation 2073/2005.

14. Data on occurrence of *E. coli* on leafy greens

Occurrence of *E. coli* on leafy greens and fresh cut leafy greens from a selection of studies published in scientific journals after 1999 are presented in Tables 4 and 5. There are difficulties in comparing these studies due to the use of different *E. coli* detection/enumeration methods which have different detection limits. In addition, some studies only detect *E. coli* at a maximum threshold level (including using the MPN technique) without determining their actual levels in the respective leafy greens. Also, when *E. coli* levels are presented in the references this is done in a heterogeneous way, i.e. either only presenting average levels or the distribution of all the observed levels according to different ranges, which may also vary among studies. Percentage of samples positive for *E. coli* ranged from 0% to 50%, with however large variations among studies in the number of samples tested and the limit of detection or enumeration. With respect to the usage of *E. coli* as a Process Hygiene Criterion (PHC)[41],

[39] EN/ISO 16649-1:2001. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of betaglucuronidase-positive *Escherichia coli* - Part 1: Colony-count technique at 44 degrees C using membranes and 5-bromo-4-chloro-3-indolyl beta-D-glucuronide. International Organization for Standardization, Geneva, Switzerland.

[40] EN/ISO 16649-2:2001. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of betaglucuronidase-positive *Escherichia coli* - Part 2: Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide. International Organization for Standardization, Geneva, Switzerland.

[41] According to the Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs, a 'Process Hygiene Criterion' is a criterion indicating the acceptable functioning of the production process. Such a criterion is not applicable to products placed on the market. It sets an indicative contamination value above which corrective actions are required in order to maintain the hygiene of the process in compliance with food law.

it is useful to consider the numbers found in positive samples of leafy greens and the point(s) in the leafy green production chain where these samples were taken. Considering only studies in EU countries, this can be summarized as follows:

- At primary production, one study in Norway found 9% of 179 samples of lettuce containing more than 10 cfu g^{-1} *E. coli*, 0.1% containing between 10^2 and 10^3 cfu g^{-1} and 0.1% containing more than 10^3 cfu g^{-1} (Loncarevic et al., 2005). Another study in Belgium found 5% of 264 samples of lettuce to contain more than 10 cfu g^{-1} *E. coli* with all the 14 samples containing between 10^1 and 10^2 cfu g^{-1} and none exceeding 100 cfu g^{-1} (Holvoet et al., 2014b) (Table 4).
- At retail level, results can be summarized as follows. In Norway, only one sample of leafy greens out of more than 300 tested (0.3%), contained more than 10 cfu g^{-1} *E. coli* (Johannessen et al., 2002). In the UK, among several thousand fresh produce samples tested over 3 studies covering various types of origin and mode of distribution, 1.4% to 13.5 % contained more than 20 cfu g^{-1} *E. coli* and 0.5% to 3% contained more than 10^2 cfu g^{-1} *E. coli* (Little and Gillespie, 2008). Very few samples (around 0.1%) contained more than 10^3 cfu g^{-1} *E. coli* (Sagoo et al., 2001, 2003a) and no samples contained more than 10^4 cfu g^{-1} *E. coli*.
- In catering establishments in the UK, prevalence of *E. coli* among 2900 samples of unpackaged vegetable salads (80% of which being leafy greens) was 3.7% of samples with 20 to 10^2 *E. coli* g^{-1}, 2% with 10^2 to 10^3 *E. coli* g^{-1}, 0.8% with 10^3 to 10^4 *E. coli* g^{-1} and 0.1% with 10^4 to 10^5 *E. coli* g^{-1}(Sagoo et al., 2003a).
- For fresh cut lettuce sampled at processing in Belgium, 9/18 samples contained between 10 and 10^2 cfu g^{-1} with 3/18 samples containing levels between 10^2 and 10^3 cfu g^{-1} (Holvoet et al., 2012) (Table 5). In Switzerland, 5/142 samples (3.5%) contained between 10^2 and 10^3 cfu g^{-1} (Althaus et al., 2012).

Relationships between the presence of generic *E. coli* and some practices in primary production or processing are not always consistent among studies, presumably because the studies involved very diverse situations. The main results are summarized below, including studies done outside Europe.

For irrigation water, the relationship between levels of indicators in water and on the irrigated produce at harvest was investigated in a study following 120 farms in the US, using different irrigation systems (sprinkler and drip irrigation) and water of different origins (surface water or ground water). The waters had wide differences in numbers of *E. coli* (from undetectable to 10^4/100 ml) with surface water more contaminated than ground water (Won et al., 2013b). At harvest, no relationship was found between the numbers of *E. coli* on leafy greens and the numbers of *E. coli* in the irrigation water. For example, some samples of leafy greens contained high numbers of *E. coli* (2 to 4.5 log cfu g^{-1}), whereas no *E. coli* was detected in the water used to irrigate these samples (Won et al., 2013b). The authors also compiled data from two previous studies done in the EU with lettuce irrigated by waste water, which found no correlation between indicators (faecal coliforms, total coliforms, faecal streptococci) in irrigation water and on the harvested lettuce.

For the use of manure as fertilizer, the percentage of *E. coli* positive leafy green samples varied widely among 40 farms in the US with diverse fertilization practices, and a significant association between the use of manure aged less than one year and the highest percentage of *E. coli* positive samples was observed (Mukherjee et al., 2004). In contrast, in experimental fields, comparing lettuce grown on soil amended with inorganic fertilizer, compost, manure or slurry, Johannessen et al. (2004) found no difference in the numbers and percentage of lettuce samples positive for *E. coli* at harvest. This could be explained by the observation that although high numbers of *E. coli* originated from the manure (around 10^5 cfu g^{-1}), *E. coli* rapidly declined and was present in low numbers in the soil at the time of lettuce harvesting (Johannessen et al., 2005). This is consistent with the study of Park et al. (2013) which did not find manure as a significant risk factor for contamination of spinach with generic *E. coli*. Park et al. (2013) found irrigation with pond water and the proximity of a poultry farm as significant risk factors. In a survey concerning several catering establishments in the UK, Sagoo et al. (2003a) noted that although one third of salads were manipulated with bare hands and one third

displayed salads at temperature above 8°C there was no relationship between the numbers of *E. coli* found and the different practices.

In conclusion, considering the studies cited above, between 50% and 99.7% of leafy greens sampled in the EU contained less than 10 *E. coli* g^{-1}, between 0% and 16% contained more than 10^2 *E. coli* g^{-1}, and between 0% and 0.8% contained more than 10^3 *E. coli* g^{-1}. Relationships between primary production practices and numbers of *E. coli* on leafy greens at harvest were unclear in all studies. One possible reason is that *E. coli* derived from irrigation water or manure declined and was no longer present at harvest. Furthermore, there are wide variations in the conditions found in surface water (canals or reservoirs) used for irrigation and the numbers as well as the rates of decline of *E. coli* are extremely variable (Won et al., 2013a), consequently it is difficult to establish a general relationship between the occurrence and levels of *E. coli* in the production environment and the occurrence and levels of *E. coli* in leafy greens at the time of harvest. However, because *E. coli* is not often detected on leafy greens, is present in high numbers in faecal material (*e.g.* fresh manure) and declines in the soil or on leafy greens during primary production, it can be considered as an indicator of a recent exposure to risk factors for *Salmonella* (e.g. flooding as observed by Castro-Ibañez et al. (2013)). *E. coli* is not suitable as an indicator for Norovirus contamination in shellfish (Lees, 2000) however there is insufficient information to establish if this is also true in other food types including leafy greens.

Table 4: Occurrence of *E. coli* on whole leafy greens

Sampling place	Commodity	Country	Detection method	n	%	95% CI[(a)]	Detection limit	*E. coli* levels	Reference
Farm	lettuce	Belgium	RAPID'*E. coli* 2/Agar (BioRad, France)	264	5.0	[2.8,8]	> 5 CFU/g	14/264 samples contained numbers with 10-100 CFU/g with none > 100 CFU/g	(Holvoet et al., 2014b)
Farm	lettuce (romaine, batavia, trocadero, iceberg, maravella)	Spain	ISO 7251:2005 (MPN)	144	17.4	[11.9,24.2]	> 30 MPN/100g	Conventional farms: 9 positive samples out of 72 (1.4% with 30-99 MPN/100g, 2.8% with 100-999 MPN/100g and 8.4% with >1000 MPN/100g) Organic farms: 16 positive samples out of 72 (13.9% with 30-99 MPN/100g, 8.3% with 100-999 MPN/100g and 0% with >1000 MPN/100g)	(Oliveira et al., 2010b)
Farm	lettuce	Norway	NMKL 1996 no.125 (enumeration of thermotolerant coliform bacteria including further identification of *E. coli*)	179	8.9	[5.4,13.8]	> 10 CFU/g	Out of 16 positive samples: 12 < 100 CFU /g, 4 ≥ 100 CFU /g (namely 100, 120, 1700 and 5000 CFU /g respectively).	(Loncarevic et al., 2005)
Farm[42]	leafy greens (kale, spinach, amaranth, Swiss chard)	US	MPN method: broth cultures from positive tubes were streaked on eosin	88	14.8	[8.5,23.3]	NA	ND	(Mukherjee et al., 2004)
	lettuce	US	methylene blue (EMB;	55	22.4	[12.5,34]	NA	ND	(Mukherjee et al., 2004)
	cabbage	US	Neogen) plates. Suspect *E. coli* colonies were confirmed with biochemical tests and Analytical Profile Index (API 20E) strips (bioMerieux, Marcy l'Etoile, France).	54	10.2	[4.8,21.5]	NA	ND	(Mukherjee et al., 2004)
Farm[43]	leafy greens	US	Three tube most-probable-number (MPN) system	296	14.9	[11.2,19.3]	NA	Out of 296 samples: 44 positive samples 2.2 to 2.4 log MPN/g	(Mukherjee et al., 2006)
	lettuce	US	using three 10-fold dilutions in 9-ml tubes of	157	15.9	[10.8,22.2]	NA	Out of 157 samples: 25 positive samples 2.2 to 2.4 log MPN/g	(Mukherjee et al., 2006)
	cabbages	US	LST broth	198	9.1	[5.7,13.7]	NA	Out of 198 samples: 18 positive samples 2.2 to 2.4 log MPN/g	(Mukherjee et al., 2006)

[42] This study includes sampling at both organic and conventional farms.
[43] This study includes sampling at organic, semi-organic and conventional farms.

Sampling place	Commodity	Country	Detection method	n	%	95% CI[(a)]	Detection limit	*E. coli* levels	Reference
Farms and packing sheds	cabbage	US	3M Coliform/*E. coli* Petrifilm™	58	29.0	[18.8,41.8]	> 5 CFU/g	Mean *E. coli* concentration: 1.1 ± 0.09 log CFU/g	(Ailes et al., 2008)
	collards	US		27	0	[0,8.8]	> 5 CFU/g	Mean *E. coli* concentration: 0.7 ± 0.00 log CFU/g	(Ailes et al., 2008)
	kale	US		9	0	[0,23.8]	> 5 CFU/g	Mean *E. coli* concentration: 0.7 ± 0.00 log CFU/g	(Ailes et al., 2008)
	arugula	US		15	0	[0,15.2]	> 5 CFU/g	Mean *E. coli* concentration: 0.7 ± 0.00 log CFU/g	(Ailes et al., 2008)
	spinach	US		27	0	[0,8.8]	> 5 CFU/g	Mean *E. coli* concentration: 0.7 ± 0.00 log CFU/g	(Ailes et al., 2008)
Farmers' and public markets	lettuce	Canada	Health Canada procedure MFHPB-19 ''Enumeration of Coliforms, Fecal Coliforms and *E. coli* in Foods using the MPN Method'' with modifications to analyze samples for *E. coli.*	128	18.0	[12.1,25.3]	NA	23 positive samples with average count of 1.25 log MPN/g	(Bohaychuk et al., 2009)
	spinach	Canada		59	27.1	[17.1,39.4]	NA	16 positive samples with average count of 1.54 log MPN/g	(Bohaychuk et al., 2009)
Retail distribution centres and farms	head lettuce	Canada	3M Coliform/*E. coli* Petrifilm™	155	0	[0,1.6]	> 5 CFU/g	Out of 155 samples: 0% positive (<5 CFU/g)	(Arthur et al., 2007)
	leaf lettuce conventional	Canada		263	6.5	[4,9.9]	> 5 CFU/g	Out of 263 samples: 17 positive (6.5%) (range: <5-260 CFU/g)	(Arthur et al., 2007)
	leaf lettuce organic	Canada		112	11.6	[6.7,18.5]	> 5 CFU/g	Out of 112 samples: 13 positive (11.6%) (range: <5 CFU/g -290 CFU/g)	(Arthur et al., 2007)
Local retail	lettuce	UK	ISO/CEN 16649- β-glucuronidase reaction.	151	0	[0,1.6]	> 20 CFU/g	Out of 151 samples: 0% positive (<20 CFU/g)	(Little et al., 1999)
Retail	ready-to-eat organic vegetables (i.e. cabbage, lettuce, watercress, cress, spinach, chard)[44]	UK	PHLS Standard Method for Food Products F17	3198	1.5	[1.1,2]	> 20 CFU/g	Out of 48 positive samples: 37 samples with 20 to $<10^2$ CFU /g, 9 samples with 10^2 CFU /g to $<10^3$ CFU /g and 2 samples with 10^3 CFU /g to $<10^4$ CFU /g.	(Sagoo et al., 2001)
Retail	total	Spain	ISO 7251:2005 (MPN)	28	7.1	[1.5,21]	> 10 CFU/g	ND	(Abadias et al., 2008)
	iceberg	Spain		5	0	[0,37.9]	> 10 CFU/g	ND	(Abadias et al., 2008)
	lettuce hearts	Spain		3	0	[0,53.6]	> 10 CFU/g	ND	(Abadias et al., 2008)

[44] The figures presented by this study also include the outcome of sampling of other vegetables (broccoli, carrot, cauliflower, celeriac, celery, mushrooms, radish, spring onions, cucumber, pepper, tomato, baby corn, cherry tomato, leeks, shallots).

Sampling place	Commodity	Country	Detection method	n	%	95% CI[(a)]	Detection limit	*E. coli* levels	Reference
	oakleaf	Spain		5	0	[0,37.9]	> 10 CFU/g	ND	(Abadias et al., 2008)
	trocadero	Spain		5	20.0	[2.3,62.9]	> 10 CFU/g	ND	(Abadias et al., 2008)
	romaine	Spain		5	0	[0,37.9]	> 10 CFU/g	ND	(Abadias et al., 2008)
	endive	Spain		5	20.0	[2.3,62.9]	> 10 CFU/g	ND	(Abadias et al., 2008)

(a): The credible interval was calculated using a Bayesian approach and taking as prior beta (1/2,1/2) (Miconnet et al., 2005).
ND: not determined
NA: not available

Table 5: Occurrence of *E. coli* on fresh-cut leafy greens

Sampling place	Commodity	Country	Detection method	n	%	95% CI [(a)]	Detection Limit	Observed *E. coli* levels	Reference
Before and after processing (washing and packing)	savoy (curly leaves) and baby (flat leaves) spinach	US	3M Petrifilm™ *E. coli* count plates	1356	8.9	[7.5,10.5]	> 10 CFU/g	Out of 122 positive samples: 51 samples with >10 CFU/g to 10^2 CFU/g, 38 samples with >10^2 CFU/g to 10^3 CFU/g and 33 samples with >10^3 CFU/g.	(Ilic et al., 2008)
End processing	fresh-cut leafy vegetables i.e. radicchio, sugarloaf, curled endive, lettuce[45]	Belgium	RAPID'*E. coli* 2 agar (BioRad, USA)	18	16.6	[28.4,71.6]	> 10 CFU/g	Out of 9 positive samples: 6 samples with >10 CFU/g to 10^2 CFU/g, 3 samples with >10^2 CFU/g to 10^3 CFU/g	(Holvoet et al., 2012)
Sampling at the production plant level	ready-to eat lettuce	Switzerland	ISO 16649-2:2004[46]	142	3.5	[1.4,7.5]	100 CFU/g	5 samples ranging between 10^2 CFU/g to 10^3 CFU/g	(Althaus et al., 2012)
Retail	total	Spain	ISO 7251:2005 (MPN)	65	7.7	[3,16]	> 10 CFU/g	ND	(Abadias et al., 2008)
	arugula	Spain		5	40	[9.4,79.1]	> 10 CFU/g	ND	(Abadias et al., 2008)
	endive	Spain		21	0	[0,11.1]	> 10 CFU/g	ND	(Abadias et al., 2008)
	lettuce	Spain		29	3.4	[0.4,15]	> 10 CFU/g	ND	(Abadias et al., 2008)
	spinach	Spain		10	20.0	[4.4,50.3]	> 10 CFU/g	ND	(Abadias et al., 2008)
Supermarkets	leafy vegetables and mixes (i.e. lettuce, collard greens, arugula, watercress, chicory, escarole, spinach, Swiss chards, colewort)[47]	Brazil	3M Petrifilm™ *E. coli* count plates	512	2.8	[1.6,4.4]	> 10 CFU/g	498 samples with <10^2 CFU/g, 8 samples with10^2 CFU/g to <10^3 CFU/g, 3 samples with with10^3 CFU/g to <10^4 CFU/g and 3 samples with with10^4 CFU/g to <10^5 CFU/g.	(Sant'Ana et al., 2011)

[45] The figures presented by this study also include the results of samples of other vegetables (parsley and chives)

[46] EN/ISO 16649-2:2001. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of betaglucuronidase-positive *Escherichia coli* - Part 2: Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide. International Organization for Standardization, Geneva, Switzerland.

[47] The figures presented by this study also include the results of samples of other vegetables and salad mixes including carrots, tomatoes, cauliflower, broccoli, onion, green pepper, leek.

Sampling place	Commodity	Country	Detection method	n	%	95% CI [(a)]	Detection Limit	Observed *E. coli* levels	Reference
Supermarkets (minimally processed leafy vegetable samples)	arugula	Brazil	BAM Standard Method (MPN)	3	50	[17.7,96.1]	MPN method	Not specified for the different leafy greens in MPN/g	(Oliveira et al., 2011)
	spinach	Brazil		6	66.7	[28.6,92.3]	MPN method		
	wild chicory	Brazil		6	46.2	[16.7,83.3]	MPN method		
	chicory	Brazil		7	63.3	[23.5,86.1]	MPN method		
	cabbage	Brazil		14	50	[25.9,74.1]	MPN method		
	Chinese cabbage	Brazil		2	15.4	[0,66.7]	MPN method		
	kale	Brazil		21	70	[50.3,87.1]	MPN method		
	lettuce	Brazil		5	19.2	[2.3,62.9]	MPN method		
	watercress	Brazil		1	25	[0,85.3]	MPN method		

ND: not determined

15. Microbiological criteria for leafy greens

15.1. Food safety assurance in leafy greens production

EU Food hygiene legislation (Regulation (EC) No 852/2004) lays down minimum hygiene requirements; official controls are in place to check food business operators' compliance and food business operators should establish and operate food safety programs and procedures based on HACCP principles. EC No 2073/2005 on microbiological criteria (MC) for foodstuffs is a Regulation of the food hygiene legislation applicable since January 2006. It is important to emphasize that the safety of food is predominantly ensured by a preventive approach, such as implementation of Good Agricultural Practices (GAP), Good Manufacturing Practices (GMP), Good Hygiene Practices (GHP) and application of procedures based on HACCP principles while microbiological criteria can be used for validation and verification of these procedures. This is also the main principle in the legislation.

In fresh-cut companies, sampling plans for microbiological testing of the end product should be in place according to Regulation (EC) 852/2004 (Article 4) and criteria for certain ready-to-eat products are set out in Regulation (EC) 2073/2005 as amended. Results of industry testing are not generally available and not centrally collected at the EU level. In France the fresh-cut industry collected testing results for *Salmonella* between 2010 and 2012 and found no positive samples from more than 1,000 samples tested. Microbiological testing of irrigation water can also be undertaken (Appendix A, Freshfel, 2013), following a more formal sampling plan for leafy greens intended for the fresh-cut industry depending on type of leafy greens, source of water, mode of irrigation. Results of irrigation water testing are not reported.

In the European Union legislation, in relation to leafy greens, microbiological criteria have been established only for pre-cut RTE vegetables (see 15.2.1. and 15.2.2.).

15.1.1. Summary of the most important preventive measures at primary production and during processing and marketing

The most important preventive measures at primary production are included in the Good Agricultural Practices (GAP). The preventive measures should focus on identified routes of microbial contamination and they should be science and risk-based. Briefly, key factors that should be monitored to reduce the microbial risk associated with leafy greens should focus on monitoring worker health, the practice of good personnel hygiene, the use of safe agricultural water (for irrigation and pesticide application), the proper composting and observance of waiting times with respect to the use of animal-derived soil amendments and the monitoring and protection of fields from faecal contamination from animals including birds.

To reduce microbial risk relating to irrigation water, it is recommended that water systems are inspected on a regular basis including the water source, distribution system, facilities and equipment. Depending on the type of water source and method of irrigation, microbial sampling may be recommended at different frequencies. There is no widespread agreement regarding the microbiological guidelines to be established for irrigation water but they should be preferably based upon risk assessment as recommended in WHO documents (http://www.who.int/water_sanitation_health/dwq/gdwq3rev/en/). An example of implementation is to be found in the Australian Water guidelines (http://www.environment.gov.au/resource/national-water-quality-management-strategy-australian-guidelines-water-recycling-managing-0) and this may vary depending upon the time between irrigation and harvest and the type of irrigation method (Fonseca et al., 2011; Ottoson et al., 2011). In most cases, the enumeration of generic *E. coli* is used as an indicator organism as its presence relates to faecal pollution or failures in control measures.

Direct or indirect contact between manure and fresh leafy greens should always be excluded. Proper composting, storage and management of organic fertilizers are essential. It is good practice to maximize the time interval between the soil amendment application and time to harvest; and soil

amendment application techniques must control, reduce or eliminate the likely contamination of surface water and/or edible crops being grown.

Animal intrusion should be minimized and growers should monitor this during growing season and immediately prior to harvest and if it happens, they should evaluate whether to harvest. If animals are allowed to graze, an adequate waiting period should be established before fields are used for cultivation of leafy greens.

The most important preventive measures during processing are included in Good Manufacturing Practices (GMP) and Good Hygiene Practices (GHP). Briefly, key factors that should be monitored to reduce the microbial risk associated with the processing of leafy greens should focus on verification of requirements for incoming products, worker health and personnel hygiene and water management.

Packers should keep all current information concerning each lot such as information on incoming materials (e.g. information from growers, lot numbers), data on the quality of water used at harvest or on farm post-harvest, pest control programmes, cooling and storage temperatures, agricultural chemicals, and cleaning schedules for premises, facilities, equipment and containers (CAC, 2003).

Food business operators should validate the quality and safety of the products by verification of the records of production and distribution. It is recommended to keep the records over an extended time period to facilitate a recall and foodborne illness investigation, if required. This period could be much longer than the shelf life of fresh fruits and vegetables. Documentation can enhance the credibility and effectiveness of the food safety management system.

Training should be provided for all personnel, including temporary/seasonal and part time workers, involved in all stages of the leafy greens supply chain from farm to fork. Awareness of food borne diseases and *Salmonella* and Norovirus as relevant microbial hazards and their transmission routes to leafy greens should be raised. In addition, workers should be trained to recognize the symptoms of diarrheal illness and be instructed on what to do if they get sick. Record keeping of these training initiatives is recommended.

15.2. Introduction to microbiological criteria

A microbiological criterion consists of specific elements such as the analytical method, the sampling plan, microbiological limit(s), and the specified point of the food chain where the limit(s) apply, the number of analytical units that should confirm to the limit(s) and the actions to be taken when the criterion is not met. Microbiological criteria should be scientifically based and are also used as a way to communicate the level of hazard control that should be achieved. Meeting microbiological criteria offers some assurance that particular pathogens are not present at unacceptably high concentrations, but does not guarantee 'absence' of those pathogens.

Microbiological criteria are essential for validation and verification of HACCP-based processes and procedures as well as Good Agricultural Practices (GAP), Good Hygiene Practices (GHP) and Good Manufacturing Practices (GMP). In addition, microbiological criteria are used to assess the acceptability of a batch of food, including the circumstances where there is insufficient knowledge of production conditions e.g. at port of entry. The microbiological criteria do not mean that all food batches have to be tested, but they clarify how the test results should be interpreted from a food batch, and the risk management consequences (EFSA, 2007a).

Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs introduces two different types of criteria; Food Safety Criteria and Process Hygiene Criteria. A Food Safety Criterion is defined in the EU-legislation as a criterion defining the acceptability of a product or a batch of foodstuff applicable to products placed on the market. If a Food Safety Criterion is not met for a product or batch of foodstuff, then this should not be placed on the market or, if it already has, be considered for recall. A Process Hygiene Criterion is defined as a criterion indicating the acceptable functioning of the production process. Such a criterion is not applicable to products placed on the

market. It sets an indicative contamination value above which corrective actions are required in order to maintain the hygiene of the process in compliance with food law. A Process Hygiene Criterion communicates the expected outcome of a process as end of manufacturing or end product criteria. They define the expected final outcome of the processes, but they neither characterize nor differentiate between the processes themselves (EFSA, 2007a). If a Process Hygiene Criterion is not met by the food business operator, corrective actions are required in order to maintain the hygiene of the process in accordance with the legislation.

The current legal framework does not include microbiological criteria applicable at the primary production stage. It is here proposed to define criteria to validate and verify Good Agricultural Practices (GAP) and Good Hygiene Practices (GHP). These criteria will be designated as Hygiene Criteria and are defined as criteria indicating the acceptable functioning at pre-harvest, harvest and on farm post-harvest production prior to processing. Hygiene Criteria should be considered as distinct from Process Hygiene Criteria, which are applicable to food business operators, although some or all of the minimal processing actions (cleaning, coring, peeling, chopping, slicing or dicing and washing) may be common to both primary producers as well as food business operators.

15.2.1. Hygiene Criteria for leafy greens at primary production

E. coli was identified as suitable for a Hygiene Criterion at primary production of leafy greens and could be considered for validation and verification of Good Agricultural Practices (GAP) and Good Hygiene Practices (GHP).

Establishment of such an *E. coli* Hygiene Criterion would inform the evaluation of the food safety control systems at primary production and on the basis of this evaluation, growers should take corrective actions based on the main mitigation options previously described. These mitigation options should focus on the appropriate implementation of Good Agricultural Practices (GAP) and Good Hygiene Practices (GHP) with special attention to 1) appropriate management of manure which might include aerobic composting, anaerobic digestion, aeration of sludge, and stabilization; 2) maintenance of the microbial quality of irrigation water, for which a water treatment might be necessary, 3) cleaning of contaminated equipment, and 4) strict control of the worker hygiene. In addition growers should provide information to the manager of the subsequent step in the food chain.

Although there is not always a direct association between the presence of *E. coli* and the presence of pathogens in leafy greens, application of an *E. coli* criterion is expected to have an impact on identification of the risk from pathogens being present if the limit for corrective measures is established in accordance with what is obtainable using Good Agricultural Practices (GAP) and Good Hygiene Practices (GHP). In most cases the level of *E. coli* in leafy greens, at the farm level (including whole heads and pre-cut processed product) is below 100 CFU/g (Table 4 and 5). Levels above 100, 1000 and even 10.000 CFU/g have been found in different specific studies, (see Tables 4 and 5) and may indicate failures in Good Agricultural Practices (GAP) and Good Hygiene Practices (GHP). A Hygiene Criterion should be seen in connection with all the preventive measures in place and an appropriate testing frequency should be applied. The limit of an *E. coli* Hygiene Criterion is set according to what is generally obtainable when applying Good Agricultural Practices (GAP) and Good Hygiene Practices (GHP) and is not a direct indicator of a risk. However an elevated number of *E. coli* (above the level normally observed) indicates a higher degree of exposure to faecal contamination and therefore potential exposure to pathogens such as *Salmonella* and Norovirus (see also chapter 15.2.2). *E. coli* is also suggested as an indicator microorganism for faecal contamination in irrigation water, which should be periodically tested.

Since only part of leafy green production enters further processing (e.g. whole heads) establishment of an *E. coli* Hygiene Criterion for leafy greens at pre-harvest, harvest or on farm post-harvest would be useful at the primary production stage.

15.2.2. Process Hygiene Criteria for leafy greens

As defined in the legislation, a Process Hygiene Criterion is a criterion indicating the acceptable functioning of a production process. In Regulation (EC) No 852/2004) processing is defined as any actions that substantially alter the initial product, including heating, smoking, curing, maturing, drying, marinating, extraction, extrusion or a combination of those processes. In the scope of this opinion, only minimally processed leafy greens are considered here, i.e. those where any action is applied to the initial product (e.g. cleaning, coring, peeling, chopping, slicing or dicing and washing) and which is not included above in the definition of processing. Process Hygiene Criteria are only applicable to food business operators and not to primary producers.

Both Enterobacteriaceae and *E. coli* are commonly used as microbiological indicators in Process Hygiene Criteria for many different food commodities for example in the production of certain meat and meat products, dairy products and shellfish. The acceptable figures of m, and M in an *E. coli* Process Hygiene Criterion differ and cannot be compared since the different type of products and production processes offer different possibilities for contamination, growth and inactivation.

In most of the studies the level of *E. coli* in leafy greens, at processing and retail level (pre-cut processed product) is below 100 CFU/g (Table 5). Levels above 100 CFU/g were found in different specific studies, (see Table 5) and may indicate failures in Good Hygiene Practices (GHP) or HACCP. In the current EC legislation a Process Hygiene Criterion is already established for *E. coli* (n = 5, c = 2, m = 100, M = 1000 cfu/g[48]) in pre-cut fruit and vegetables (ready-to-eat).

A Process Hygiene Criterion should be seen in connection with all the preventive measures in place (including verification of HACCP) and an appropriate testing frequency should be applied. Based on the obtained data, if specified levels of a Process Hygiene Criterion such as *E. coli* are exceeded, processors should take internal corrective actions based on the main mitigation options previously described in the Section 12 of this Opinion. These mitigation options should focus on the appropriate implementation of Good Hygiene Practices (GHP) and Good Manufacturing Practices (GMP) with special attention to 1) the control of the microbial quality of the raw material, 2) treatment and quality maintenance of washing water to reduce the build-up of microorganisms, 3) cleaning of contaminated equipment, and 4) strict control of the worker hygiene.

A Process Hygiene Criterion for *E. coli* in leafy green packaging plants or fresh cutting plants will give an indication of the degree to which collectively GAP, GHP, GMP or HACCP programs have been implemented.

15.2.3. Food Safety Criteria for leafy greens

The EU Food Safety Criteria defined in EU legislation are for the microbiological acceptability of food products. These criteria apply to products at the end of production or placed on the market. If the criteria are not met the product/batch is expected to be withdrawn from the market. The following conclusion on Food Safety Criteria were previously stated (EFSA, 2007a):

(a) An advantage of establishing Food Safety Criteria for pathogenic microorganisms is that harmonised standards on the acceptability of food are provided for both authorities and industry within the EU and for products imported from third countries.

(b) Food Safety Criteria will impact the entire food chain, as they are set for products placed on the market. Risk of recalls and the economic loss as well as loss of consumer confidence will be a strong motivation to meet the criteria. Therefore Food Safety Criteria are assumed to have an effect on food safety and public health where there is an actual or perceived risk. However,

[48] For a given sampling plan, n = number of units comprising the sample, c = number of sample units which can give values between m and M. Interpretation of results is based on: satisfactory, values of < m; limits of acceptable with c samples giving values between m and M and the rest of values observed are ≤ m; and unsatisfactory for values of ≥ M.

it is not possible to evaluate the extent of public health protection provided by a specific Food Safety Criterion.

(c) Microbiological testing alone may convey a false sense of security due to the statistical limitation of sampling plans, particularly in the cases where the hazard presents an unacceptable risk at low concentrations and/or low and variable prevalence.

(d) Food safety is a result of several factors. Microbiological criteria should not be considered without other aspects of EU Food legislation, in particular HACCP principles and official controls to audit food business operators' compliance.

In order to establish Food Safety Criteria, it is a prerequisite that methods to properly detect the hazard are available at a reasonable cost. Inherent in this is that hazards must be accurately defined, or the result may be that food batches are erroneously considered unsafe. Regulation (EC) No 2073/2005 on microbiological criteria does not prescribe any sampling/testing frequencies except for minced meat, mechanically separated meat and meat preparations. While this leaves flexibility to tailor the intensity of testing according to the risk, it also leaves the possibility of inconsistency in testing and control (EFSA, 2007a).

In the EC legislation, a Food Safety Criterion has been established for *Salmonella* (n= 5, c= 0, absence/25g) in pre-cut fruit and vegetables (ready-to- eat). Although the prevalence of *Salmonella* in leafy green is generally below 1 % (see chapter 11) and therefore the cost-effectiveness of random testing is very low, a Food Safety Criterion for both pre-cut bagged leafy greens and whole heads or baby- or multileaves marketed without further processing, if eaten raw as salad, could be considered. A Food Safety Criterion for *Salmonella* in leafy greens intended to be eaten raw as salads could be used as a tool to communicate to producers and processors that *Salmonella* should not be present in the product. Since the prevalence of *Salmonella* is likely to be low, testing of leafy greens for this bacterium could be limited to instances where other factors indicate breaches in GAP, GHP, GMP or HACCP programs.

Noroviruses can be detected in leafy greens, but prevalence studies are limited, and quantitative data on viral load are scarce making establishment of microbiological criteria for these foods difficult. Information is lacking on the relationships between the occurrence of Norovirus as detected by real time RT-PCR, infectivity and the actual risk to public health. Real-time RT-PCR might overestimate the presence of infectious Norovirus, as it detects genomic material from infectious as well as non-infectious particles (Baert et al., 2011). For this reason a Food Safety Criterion for Norovirus in leafy greens is not recommended, and it may be necessary to acquire more data on occurrence and levels including information about any correlation between virus level and features indicative of a risk of infection.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

- Leafy greens are defined as leaves, stems and shoots from various leafy plants which are eaten as vegetables, and for the purposes of this opinion, only those eaten raw will be considered.
- The major crop types of leafy greens are: 'lettuce' types, leafy brassicas, cabbage, Belgian endive and watercress.
- 'Lettuce'-type leafy greens can be harvested at different development states, e.g. as mature whole heads, as baby leaves or as multi-leaves.
- Leafy greens may be processed to obtain ready-to-eat products, and these steps include: selection, elimination of external leaves, cutting, cooling, washing, rinsing, dewatering, packaging and storage. Other types of processing (e.g. freezing, mashing and unpasteurized juicing, blending) are either never or very rarely used and are not further considered. Some of these products are subject to cooking, pickling and other processes but these are also outside the scope of this Opinion.
- Harvested leafy greens are not subjected to physical interventions that completely eliminate microbial contamination. Technologies currently available for use by the leafy greens industry fall short of being able to guarantee an absence of *Salmonella* or Norovirus on leafy greens at primary production.

Answers to the terms of Reference

TOR 3. To identify the main risk factors for the specific food/pathogen combinations identified under ToR 2, including agricultural production systems, origin and further processing.

- The main risk factors for the contamination of leafy greens with *Salmonella* at primary production are diverse and include:
  - Environmental factors, in particular proximity to animal rearing operations, seasonality and associated climatic conditions (e.g. heavy rainfall causing floods) that increase the transfer of pathogens from their reservoirs;
  - Contact with animal reservoirs (domestic or wild life);
  - Use of untreated or insufficiently treated manure or compost;
  - Use of contaminated agricultural water (for irrigation or pesticide treatments);
  - Cross-contamination by food handlers and equipment at harvest or on farm post-harvest.
- *Salmonella* tends to decline on the surface of leafy greens during primary production. Therefore contamination events close to harvest (e.g. by irrigation water, floods), at harvest (e.g. by food handlers) or on farm post-harvest (e.g. by cross-contamination via water or from equipment or by food handlers) are the most important risk factors at primary production.
- Internalization in leafy greens has been observed after artificial inoculation of high levels of *Salmonella* making it difficult to assess its importance under natural conditions.
- The main risk factors for the contamination of leafy greens with Norovirus at primary production are diverse and include:

  - Environmental factors, in particular climatic conditions (e.g. heavy rainfall or floods) that increase the transfer of Norovirus from sewage or sewage effluents to irrigation water sources or fields of leafy greens;

  - Use of water for irrigation or pesticide treatment which has been contaminated by sewage;

  - Contamination by food handlers or equipment at harvest or on farm post-harvest.

- Internalisation of Norovirus, or surrogate viruses, in plant tissues has been observed in experimental studies. However, the virus levels used in these experimental studies may be higher than those which could be encountered during crop production; furthermore, information on Norovirus internalisation gained through the use of surrogates should be interpreted with caution, as properties of different viruses may affect uptake into, or clearance from, plants.

- For both *Salmonella* and Norovirus, processes at primary production which wet the edible portions of the crop represent the highest risk and these include spraying prior to harvest, direct application of fertilizers, pesticides and other agricultural chemicals and overhead irrigation. Subsurface or drip irrigation which results in no wetting of the edible portions of the plants are of lower risk.

- During processing, water submersion of fresh-cut leafy greens in washing tanks presents a risk of cross-contamination. For *Salmonella*, this risk is reduced if disinfectants are properly used within the washing tank water. There are few studies with surrogate viruses, such as Murine Norovirus, that investigate the effectiveness of chemical inactivation of Norovirus in processing water. The effectiveness of chlorine against Norovirus is not fully defined due to the lack of an infectivity assay.

- During processing, contamination or cross-contamination via equipment, water or by food handlers are the main risk factors for contamination of leafy greens for both *Salmonella* and Norovirus.

- Adherence or biofilm formation of *Salmonella* on processing equipment may become a source of contamination for leafy greens and may be difficult to remove by routine cleaning methods.

- At distribution, retail, catering and in domestic or commercial environments, cross-contamination of items, in particular via direct or indirect contact between raw contaminated food of animal origin and leafy greens are the main risk factors for *Salmonella*.

- At distribution, retail, catering, in domestic and commercial environments, the Norovirus-infected food handler is the main risk factor. Although less documented than for Norovirus, contamination of leafy greens with *Salmonella* by food handlers is a potential risk.

- Norovirus can persist on leafy greens. Survival of *Salmonella* can occur on leafy greens and, under certain conditions of storage growth may occur especially on fresh-cut leafy greens.

TOR 4. To recommend possible specific mitigating options and to assess their effectiveness and efficiency to reduce the risk for humans posed by food/pathogen combinations identified under ToR 2.

- Appropriate implementation of food safety management systems including Good Agricultural Practices (GAP), Good Hygiene Practices (GHP) and Good Manufacturing Practices (GMP) should be the primary objective of operators producing leafy greens eaten raw as salads. These

food safety management systems should be implemented along the farm to fork continuum and will be applicable to the control of a range of microbiological hazards.

- As *Salmonella* has reservoirs in domestic as well as wild animals, birds and humans, the main mitigation options for reducing the risk of contamination of leafy greens are to prevent direct contact with faeces as well as indirect contact through slurries, sewage, sewage sludge, and contaminated soil, water, equipment or food contact surfaces.

- Compliance with hygiene requirements, in particular hand hygiene, is an absolute necessity for food handlers at all stages of the leafy green production and supply chain to reduce the risks of both *Salmonella* and Norovirus contamination.

- Production areas should be evaluated for hazards that may compromise hygiene and food safety, particularly to identify potential sources of faecal contamination. If the evaluation concludes that contamination in a specific area is at levels that may compromise the safety of crops, in the event of heavy rainfall and flooding for example, intervention strategies should be applied to restrict growers from using this land for primary production until the hazards have been addressed.

- Each farm environment (including open field or greenhouse production) should be evaluated independently as it represents a unique combination of numerous characteristics that can influence occurrence and persistence of pathogens in or near fields of leafy greens.

- Among the potential interventions, both water treatment and efficient drainage systems that take up excess overflows are needed to prevent the additional dissemination of contaminated water. Since *E. coli* is an indicator microorganism for faecal contamination in irrigation water, growers should arrange for periodic testing to be carried out to inform preventive measures.

- All persons involved in the handling of leafy greens should receive hygiene training appropriate to their tasks and receive periodic assessment while performing their duties to ensure tasks are being completed with due regard to good hygiene and hygienic practices.

- Clear information (including labelling) should be provided to consumers on appropriate handling of leafy greens which includes specific directions for product storage, preparation, intended use, 'use-by' date or other shelf-life indicators.

TOR 5. To recommend, if considered relevant, microbiological criteria for the identified specific food/pathogen combinations throughout the production chain.

- The current legal framework does not include microbiological criteria applicable at the primary production stage. It is here proposed to define criteria to validate and verify Good Agricultural Practices (GAP) and Good Hygiene Practices (GHP). These criteria will be designated as Hygiene Criteria and are defined as criteria indicating the acceptable functioning at pre-harvest, harvest and on farm post-harvest production prior to processing.

- Hygiene Criteria should be considered as distinct from Process Hygiene Criteria, which are applicable to food business operators, although some or all of the minimal processing actions (cleaning, coring, peeling, chopping, slicing or dicing and washing) may be common to both primary producers as well as food business operators.

- *E. coli* was identified as suitable for a Hygiene Criterion at primary production of leafy greens and could be considered for validation and verification of Good Agricultural Practices (GAP) and Good Hygiene Practices (GHP) and on the basis of this, growers should take appropriate corrective actions.

- A Process Hygiene Criterion for *E. coli* in leafy green packaging plants or fresh cutting plants will give an indication of the degree to which collectively GAP, GHP, GMP or HACCP programs have been implemented.

- A Food Safety Criterion for *Salmonella* in leafy greens intended to be eaten raw as salads could be used as a tool to communicate to producers and processors that *Salmonella* should not be present in the product.

- Testing of leafy greens for *Salmonella* could be limited to instances where other factors indicate breaches in GAP, GHP, GMP or HACCP programs.

- Noroviruses can be detected in leafy greens, but prevalence studies are limited, and quantitative data on viral load are scarce making establishment of microbiological criteria for these foods difficult.

- Information is lacking on the relationships between the occurrence of Norovirus as detected by real time RT-PCR, infectivity and the actual risk to public health.

RECOMMENDATIONS

- There should be implementation and evaluation of procedures such as sanitary surveys, training, observational audits and other methods to verify hygiene practices for leafy greens.

- Further data should be collected to support *E. coli* criteria at both primary production and during processing of leafy greens. This should also include standardization of sampling procedures at primary production.

- A more detailed categorisation of food of non-animal origin should be introduced to allow disaggregation of the currently reported data collected via EFSA's Zoonoses database on prevalence and enumeration of foodborne pathogens.

- Risk assessment studies are needed to define the level of hazard control that should be achieved at different stages of production systems. Such studies should be supported by targeted surveys on the occurrence of *Salmonella* and Norovirus at specific steps in the food chain.

- ISO methods and technical specifications (including for alternative methods) for Norovirus detection in leafy greens should be further refined with regard to sampling, sample preparation, limit of detection and interpretation of results.

- Research should be undertaken with the aim of: a) developing infectivity assays for Norovirus and b) understanding the extent of *Salmonella* and Norovirus internalisation in plant tissue during crop production at natural exposure levels.

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APPENDICES

Appendix A. List of questions to be addressed by the European Fresh Produce Association (Freshfel) and information received from Freshfel on 22 July 2013

List of questions to be addressed by the European Fresh Produce Association (Freshfel)

1. How do you categorise 'leafy greens to be eaten raw as salads' according to different:
   - production systems,
   - processing (excluding thermal treatment or any equivalent (e.g. blanching as well as shelf stable juices) and
   - presentation at retail?

   All questions listed below aim at characterizing the 'leafy greens sector' in the EU. Please note that for convenience in all questions 'leafy greens' refers to 'leafy greens to be eaten raw as salads'.

PRODUCTION SECTOR

2. Provide an overview of this sector listing the most commonly produced botanical varieties of leafy greens in the EU?
3. Which are the top 10 types of leafy greens produced in EU?
4. Which are the top 10 types of leafy greens sold in EU?
5. Which countries are the major producers in the EU?
6. Which are the main third countries providing the EU with leafy greens?
7. Which is the share of the market covered by imported production versus intra-EU production of leafy greens?
8. What is the share of leafy greens producers which are not members of Freshfel in the EU?

   Which volume of production do these producers represent?
9. Are there any figures in the EU to characterize the proportion of the production of leafy greens from 'home/small scale' producers when compared to 'large-scale' production?
10. Provide available figures on (i) production, (ii) producers, (iii) trade, (iv) certification and (v) distribution (type of outlets) of the leafy greens.

AGRICULTURAL PRODUCTION SYSTEMS

11. Are there any producer's survey results which could help to describe how leafy greens are produced in the EU?
12. Characterise the profile of workers in the production of leafy greens (e.g. training, casual workers, foreign workers etc).
13. Please indicate percentages of production of leafy greens (i) in fields, (ii) in greenhouses (iii) soilless (hydroponics) or (iv) in soil?
14. Are there any additional production systems in place in the EU (as well as for imported products)?
15. Which leafy greens can be produced as hydroponic crop?
16. Indicate the major irrigation systems and water sources in the agricultural production of leafy greens.

    Is the water quality controlled (microbiologically)? If so and if available, provide, data on microbiological quality of the water used in the agricultural production of leafy greens.

PROCESSING OF LEAFY GREENS

17. Which are the most common processing practices for leafy greens in the EU?
18. Which agricultural practices and processing steps - can be executed (i) only manually, (ii) both manually or mechanically or (iii) preferentially mechanically?

    What are the percentages of manual versus mechanical practices?
19. Indicate the major water sources in the processing of leafy greens.

    Is the water quality controlled (microbiologically)? If so and if available, provide data on microbiological quality of the water used in the processing of leafy greens.
20. How important is the share of production in the EU for (i) whole heads (ii) baby leaves (ii) multi leaves and (iv) micro veggies (micro greens)?
    Which proportion of these products are (i) sold directly (without further processing) or (ii) undergoing processing (cutting, mixing and packaging)?

DISTRIBUTION AND RETAIL

21. Which are the procedures and conditions for transport and distribution of leafy greens in the EU?
22. Are there any specific control measures in place in the EU to maintain the cold chain during storage and distribution of leafy greens?
23. Which proportion of leafy greens may be sold without temperature control during distribution in the EU?
24. Describe how traceability of leafy greens is addressed for the different agricultural production systems and processing options?

SYSTEMS IN PLACE TO ENSURE SAFETY OF PRODUCTS

25. Are there any European guidelines/codes available from Freshfel or other associations of producers on practices (including cutting and mixing) to ensure food safety in the production of leafy greens?
26. In your view, what are the strengths and weaknesses of the current Good Agricultural Practices (GAP), Good Manufacturing Practices (GMP) and standards to ensure microbiological quality of leafy greens?
27. In your view which are the major weak points from the microbiological point of view in the agricultural production systems as well as processing of leafy greens?
28. Do the producers of fresh-cut, pre-packaged leafy greens in the EU need to be registered as food processing establishments?
29. What are the hygienic requisites that these processing establishments need to comply with?

    How is compliance with these hygienic requisites verified?
30. Are there any central repositories of data on non-compliance with the Good Agricultural Practices (GAP), Good Manufacturing Practices (GMP), standards as well as on the analysis of these data?
31. Are there many companies producing leafy greens which are applying the ‘test to release’ for microbiological parameters? If so, are companies using presence/absence tests? In case enumeration testing is used, which are the threshold levels (cfu/g) used for interpretation of the analysis results?

32. Are the producers, producer associations or any other stakeholders (e.g. retail) also doing regular testing/monitoring of leafy greens?
33. Which are the sampling plans used in the scope of this testing/monitoring of leafy greens?
34. Is there any additional testing/monitoring in place for imported leafy greens?
35. Does Freshfel have any available data on levels of detection and enumeration of *Salmonella* and Norovirus in leafy greens in the EU?
36. Which methods for detection and enumeration of *Salmonella* and Norovirus on leafy greens are being used in the food chain in the EU?
37. Which are the differences on the hygienic requisites for the production of organic leafy greens when compared to conventional production?

    How is compliance with these hygienic requisites verified?
38. What are the hygienic requisites in place for imported leafy greens?

    How is compliance with these hygienic requisites verified?
39. Which chemical and/or physical decontamination methods are being used in the EU for the treatment of soil, substrates, manure or compost?
40. Which chemical and/or physical decontamination methods are being used in the EU for the treatment of water (reservoirs, irrigation systems, processing water)?
41. Describe the practices in use in the EU for chemical and/or physical decontamination of leafy greens? Which are the main methods in place in the EU?
42. Which chemical and/or physical decontamination methods are allowed in the EU among Member States?
43. Does Freshfel provide specific recommendations on methods used to reduce contamination of leafy greens by *Salmonella* and Norovirus?

Information received from the European Fresh Produce Association (Freshfel) on 22 July 2013

19 July 2013

Background information leafy greens category

Opinion EFSA-Q-2012-00238

Definitions (questions 1-2)

(1)Categorisation

A. Production

Several production systems are used depending on the specifications of the final product which need to be achieved.

- Direct drilling crops (baby leaves) / transplanting crops (whole head, endives)
- Drip irrigation / sprinkler irrigation
- Hydroponic / substrates / soil
- Open air / protected (greenhouse, polytunnels, …) / production rooms

B. Processing

Fresh

- Non-packed or packed without processing (e.g. wholehead lettuce, baby leaves)
- Washed and packed in tray (e.g. lamb lettuce)
- Unwashed and packed in trays (e.g. baby leaves)
- Wholehead unwashed and packed (e.g. endive in flow-pack, iceberg in packaging film, little-gem in trays).

Fresh-cut

- Wholehead: cutting of heads (manually or mechanically), washing, rinsing, spinning, sorting in bag, bowl or tray
- Baby leaves and lamb lettuce: same procedures, but no cutting
- Maintenance of a specific cold chain from the harvest in the field to store delivery: transport of raw materials and finished products in refrigerated lorries, post-harvest storage and pre-production in cold rooms, use of cooled washing and rinsing water, cooled atmosphere in processing rooms

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C. Retail presentation

Fresh

- Wholehead: sales either loose in the shelf, either in wooden crates, plastic crates or cardboard, either in flow-pack, either in trays (little gem), either in plastic bags (witloof)
- Baby leaves and lamb lettuce: wooden or plastic crates, filmed trays or flowpack

Fresh-cut

Exclusively in refrigerated shelves in either bags, bowls or trays

(2) Varieties

The following botanical varieties are commonly used in the production of leafy greens, they have been grouped in the following categories.

A. Lettuce

Lactuca sativa L.

- Lactuca sativa var. capitata L. (head lettuces incl. Iceberg)
- Lactuca sativa var. longifolia Lam. (romaine lettuces)
- Lactuca sativa var. crispa L. (leaf lettuces)

Cichorium endivia L.

- Cichorium endivia var. crispum Lam. (curled-leaved endives)
- Cichorium endivia var. latifolium Lam. (broad-leaved endives, escaroles)

Beta vulgaris L. var. cicla (chard)

Valerianella locusta (lamb lettuce)

Cichorium intybus L. var. Silvestre (red chicory)

Eruca sativa Mill (rucola)

Spinacea oleracea L. (spinach)

B. Leafy brassica

Brassica rapa subsp. pekinensis (Chinese cabbage)

Brassica oleracea convar. Acephalea (kale)

C. Cabbage

Brassica oleracea var. capitata L. (green/red cabbage)

Brassica oleracea var. sabauda L. (savoy cabbage)

D. Witloof (Cichorium intybus Foliosum Group)

E. Watercress (Nasturtium officinale R. Br.)

EU market (questions 3-10, 20)

(10) Detailed statistics have been prepared for the main products constituting the leafy greens category. The data provided relate to the production in the EU, imports from 3rd countries and intra-EU import flows for each product. Production data have been obtained from FAOSTAT, whereas trade data have been obtained from EUROSTAT. It should be noted that these data do not distinct product flows going to the fresh market from product flows going to processing (canned/frozen vegetables). With the exception of spinach and cabbage where about 90% of production is destined for processing, all other leafy greens are almost exclusively destined for the fresh market (incl. fresh cut). Data for chicory witloof are not considered since both FAOSTAT and EUROSTAT provide unrealistic figures due to confusion with endives and chicory.

(5-7) Leafy greens on the EU market are pre-dominantly produced in the EU and the share of imports from 3rd countries is limited (0,3%). Except for products which can be grown year-round on the same premises (e.g. cress, endives, lettuce), leafy greens are sourced from different EU regions in the course of a year. Winter production is concentrated in Southern Europe and as the season evolves gradually moves to Northern Europe or higher altitudes. Imports from 3rd countries mainly cover temporary production gaps due to climatic conditions. The main EU producing countries for lettuce are Spain, Italy, France, Germany and UK (representing 80% of production), while the main 3rd countries supplying the EU are situated in the Mediterranean rim (Egypt, Morocco, Tunisia, Turkey) as well as the USA. For chicory witloof, France (180.000 MT), the Netherlands (60.000 MT) and Belgium (40.000 MT) are the main producing countries. Cabbage and spinach are both grown more evenly throughout Europe, although Eurostat production figures for spinach are not complete.

(3-4) The most important leafy greens are Chicory witloof, Butterhead lettuce, Iceberg lettuce, Escarole, Frisée, Lamb lettuce, Baby leaves, Oakleaf lettuce, Batavia, Lollo Rossa and Spinach. As the share of imports from 3rd countries is limited, this overview generally corresponds with the importance of the product categories in sales.

(20) There are no detailed statistics available on the share of production of whole heads versus baby leaves, multi leaves (specific varieties growing more leaves than conventional varieties) and micro greens (no clear definition, closely related to cress). Volume wise the two latter categories are niche markets and represent less than 1%. In France 85% of salads are estimated to be whole heads, whereas baby leaves are estimated to represent 15%. Sales data in France and The Netherlands suggest sales of fresh unprocessed salads represent 60-70% whereas fresh-cut salads represent about 30% of the market. The market penetration of fresh-cut salads differs between Member States and is highest in the UK. The fresh-cut segment is expected to continue growing in line with the general trend for convenience food.

(9) With regard to the differentiation between commercial production and home or small-scale production, there are no reliable figures available. Data on seeds sold in retail outlets have been sought from the European Seed Association, but were not available as such. Moreover, most leafy greens for home-production would be bought as plantlets. Spinach and endives would generally be far less home-grown. Whereas home or small-scale production of vegetables is by and large considered as marginal in Western Europe, it is more prevalent in certain Eastern European countries. The economic crisis and certain trends (local produce, authenticity) may however have contributed to an increased popularity of the segment.

Agricultural production systems (questions 11-16)

(11) The leafy greens category comprises a wide range of products which in turn can be grown in several production systems which are continuously being optimised through research and innovation (see also categorisation – production systems). There are no survey results describing how leafy greens are produced in the EU.

(13-15) Leafy greens destined for processing (e.g. cabbage, spinach) are all grown in fields, whilst witloof will generally be grown in production rooms (hydroponics, roots are however grown in field). In general about 90% of production is taking place in fields, whereas 10% in greenhouses. In theory all crops can be produced as hydroponic crops, but cost considerations imply that it is only used marginally.

(16) The major irrigation systems used in agricultural production are drip irrigation and sprinkler irrigation. The main water sources include surface waters (river, lake), reservoirs supplied by well water or rain water, and well water (drinking water in case of hydroponics). In the case of products destined for the fresh market, the water quality is mostly controlled just once per year. In the case of products destined for the fresh-cut market, a control plan is required. A water assessment of each farm determines the microbiological testing frequency according to the production system, type of crop, water source, irrigation system, ... In general E. Coli, Salmonella, Streptococcus faecalis, and total coliforms are the parameters being analysed. Some operators make use of chemical decontamination techniques when allowed, further research is also carried out to consider the option of treating irrigation water.

(12) The field staff in the production of leafy greens are mainly seasonal workers from various countries depending on the production countries (e.g. South America in the case of Spain). In the packinghouse, there's a mix between national and foreign workers. The workers are trained with regard to the prevention of food safety incidents, which is generally a prerequisite in certification schemes (e.g. GlobalGAP) and national guidelines.

Processing leafy greens (questions 17-19)

(17) The processing practices in the fresh-cut segment include quality inspection raw materials, cutting and/or grading, cleaning, rinsing, drying, packing (under modified air/atmosphere). All processes take place under regulated temperature to ensure the maintenance of the cold chain.

(18) The harvest of salads is generally taking place manually, except for lamb lettuce and baby leaves which are mechanically harvested. Cutting, grading and packing can be done both manually and mechanically, whereas cleaning, rinsing and drying are carried out mechanically. It is estimated 60% of practices in the fresh-cut operations are carried out manually.

(19) The main water sources used in the processing practices are drinking water and potable well water. The water is tested according the applicable microbiological standards for potable water.

19 July 2013

Background information distribution & food safety practices

Opinion EFSA-Q-2012-00238 and 00176

Distribution & retail (questions 21-24)

(21) No particular transport and distribution conditions apply for leafy greens destined for fresh market (i.e. transport under ambient temperature), for quality reasons many operators will nevertheless try to ensure the cold chain, particular for long haul transport (<10°C). In the case of fresh-cut, transport and distribution need to take place under regulated temperature. The practices vary per country and are fixed in national legislation (BE, DE, NL: <7°C, FR: 1-4°C, IT: <6°C, SE: 2-5°C). In general operators will apply lower temperatures to optimise quality and shelf life. Some species (e.g. herbs),however, do not support such lower temperatures.

(22) The control of the cold chain will be under the responsibility of the manufacturer until the delivery, whereby the temperature will be checked during loading and unloading of the truck as well as being registered during transport. From delivery until the purchase by the consumer, the control of the cold chain will be under the responsibility of the retailer. In the case of long term storage (e.g. cabbage, carrots, onions), cabbage and carrots are stored in cold stores whereby temperature and moisture are set. Onions are stored similarly to potatoes in ventilated cold stores whereby sprout suppressants are used.

(23) All vegetables for the fresh market may be sold under ambient temperature. In general most vegetables will however be sold under regulated temperature to maintain quality and ensure longer shelf life. Fresh-cut produce may only be sold under regulated temperature (see also question 21).

(24) Traceability: see presentation

Food safety systems (questions 25-42)

(25-26) Guidelines for good hygiene practices in fresh produce are available at national level, with separate guidance for primary production, distribution & trade as well as processing (fresh-cut). All guidance documents are generic and apply to both fruit and vegetables, although they include specific provisions for certain product categories where needed.

EU guidelines are not available, private certification systems (e.g. GlobalGAP, QS, IFS, BRC, …) however provide a broader scope.

The main strength of these schemes consists in the identification of hazards and establishment of preventive measures from field to distribution. A weakness in the guidelines on primary production is the lack of attention to microbiological and emerging risks. These are however gradually being addressed.

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(27) Major weak points in agricultural production system include the irrigation with surface water, contamination by pests or animals and contact with the soil for certain salad types. The principal weak point for fresh-cut produce is a possible major rupture of the cold chain after delivery.

(28) EU Hygiene rules (Reg. 852/2004) require the registration as food processing establishment of any company producing fresh-cut produce. The hygienic requirements these companies need to comply with are provided in Annex II which are further clarified in national good hygiene practices guidelines or private certification schemes. Control of these requirements take place through control plans, internal and external audits as well as official inspections.

(29) There is no central repository of non-compliances at EU or national level. Generally companies analyse non-compliances in order to improve their practices. Some national industry associations pool microbiological test results on fresh produce as well as chlorine data to enable collective improvement actions or monitor the state of play regarding pathogens for which no microbiological criteria have been established.

(30) Positive release schemes are not used in the fresh-cut segment given the short shelf life of fresh-cut produce and the time needed for microbiological analysis.

(31) Producers and producer associations do carry out regular testing, a microbiological control plan is defined by each party involved in primary production. A retail level a random control plan is implemented.

(32) Sampling plans for microbiological testing/monitoring are defined in the legislation and are set by each food business operator on the basis of a risk analysis.

(33 and 37-38) Imported produce is treated similarly to EU produce and is not subject to additional testing or specific other hygiene requirements.

(34) Freshfel does not have centralised data available regarding the detection of Salmonella and Norovirus on leafy greens, or Salmonella, Yersinia, Shigella and Norovirus on bulb and stem vegetables and carrots.

The French fresh-cut industry association (SFPAE) collected data for Salmonella on leafy greens, from 2010 to 2012 more than 1.000 samples per year (all negative). The association is also carrying out further research regarding norovirus (results expected in 2014).

Belgium, Germany and the Netherlands have set-up a monitoring scheme for various fruit and vegetables which will be implemented in the coming months.

(35) Detection methods being used:

- Salmonella: NEN-EN-ISO 6579:2002, BRD 07/11-12/05, Rapid Salmo AES 10/4-05/04
- Norovirus: no validated method to date (research French association SFPAE)
- Shigella: NEN-EN-ISO 21567:2004
- Yersinia: NEN-EN-ISO 10273:2003

Commercial kits are sporadically used, generally companies prefer accredited methods in order to avoid discussions in case of complaints.
Commonly vegetables in the fresh-cut segment are tested on Salmonella, E. Coli and Listeria; other pathogens may be tested for on specific request of customers.

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(36) There is no difference in hygienic requirements for the production of organic versus conventional leafy greens.

(39) Decontamination methods used in primary production:

- Soil treatment: Metam-sodium, Dazomet, 1,3-Dichloropropone, steam, solarisation
- Manure treatment: composting

These treatments are primarily meant to combat pests (nematicide) and disease, and limit weed competition (herbicide). Assurance schemes generally recommend to maximise the time between manure application and harvest. GlobalGAP recommends untreated organic fertiliser should not be used from 60 days previous to the harvest season.

(40) Water treatment methods:

- Water reservoir: mostly no treatment, where allowed oxidative or copper compounds as well as chlorine
- Irrigation system: chlorydric acid
- Processing water
  - Chemical: chlorine solutions; ozone; peracetic acid
  - Physical: UV-light, ultrasound

(41) Decontamination methods of produce:

- Chemical: not available
- Physical: grading (optical and visual), recovery of foreign bodies by difference in density in the cleaning trays, leaching during the cleaning process, rinsing with drinking water

(42) Freshfel does not provide specific recommendations on methods used to reduce contamination by pathogens on fresh produce.

Key differences EU vs US fresh produce practices

- Preventive approach (GAP, GHP) EU versus curative approach US => disinfection in the field and of finished product
- Production concentrated in South West => transportation time =>longer shelf life (14-18 days vs 7-11 days in EU)
- Processing facilities near the production sites in US vs processing facilities nearby the consumer market in EU
- Transport under regulated temperature in EU vs transport with crushed ice (source of contamination) in US
- Presence of large cattle farms with flood washing systems nearby rivers which are used for irrigation in US
- Scale of operators is much larger in US vs EU
- Larger market penetration of fresh-cut produce in US vs EU

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Appendix B. Leafy greens production statistics tables (provided by Freshfel)

Table 6: Production of lettuce in metric tons (EUROSTAT)

Producing Country	2007	2008	2009	2010	2011	2012	Share 2011 (%)
Spain	947 600	-	-	809 400	868 200	880 200	36.3
Italy	485 500	467 700	330 000	627 000	483 200	-	20.2
France	347 800	316 900	-	310 600	267 400	256 300	11.2
Germany	197 800	180 900	193 900	175 200	200 100	257 600	8.4
United Kingdom	117 000	-	-	134 000	132 000	122 000	5.5
Greece	94 800	90 400	-	115 300	106 500	-	4.5
Netherlands	85 500	90 500	86 000	82 000	92 000	103 000	3.8
Portugal	-	-	-	-	70 400	54 700	2.9
Belgium	76 400	76 100	69 400	67 100	60 800	-	2.5
Austria	51 700	47 000	44 500	38 600	38 000	47 500	1.6
Sweden	26 600	28 500	28 500	24 100	25 800	-	1.1
Poland	20 500	14 400	14 800	27 800	-	-	0.0
Denmark	-	-	-	12 600	12 700	12 700	0.5
Hungary	7 600	7 500	8 400	7 900	7 600	7 200	0.3
Finland	5 000	5 800	6 400	4 500	7 000	23 600	0.3
Bulgaria	3 600	2 100	3 300	4 400	5 100	9 800	0.2
Extra-EU	2 586	1 977	3 055	5 264	4 530	3 922	0.2
Malta	3 600	3 600	3 700	4 000	4 200	4 100	0.2
Romania	1 100	1 100	1 300	2 700	1 900	2 600	0.1
Czech Republic	-	-	-	-	1 500	1 400	0.1
Cyprus	1 500	1 700	1 400	1 500	1 100	1 100	0.0
Luxembourg	200	200	100	100	200	200	0.0
Lithuania	200	300	300	1 400	-	3 000	0.0
Slovenia	7 000	8 500	8 700	-	-	8 900	0.0
Slovakia	200	500	400	-	-	200	0.0
Estonia	-	-	-	-	-	-	0.0
Latvia	10 700	100	100	-	-	-	0.0
Ireland	-	-	-	-	-	-	0.0
Total	**2 494 486**	**1 345 777**	**804 255**	**2 455 464**	**2 390 230**	**1 800 022**	**100.0**

Table 7: Imports of lettuce from outside the EU in metric tons (EUROSTAT)

Exporting Country	2007	2008	2009	2010	2011	2012	Share 2012 (%)
Tunisia	-	116	1 386	1 563	2 374	1 803	46.0
USA	1 691	366	68	1 254	42	943	24.1
Morocco	104	204	666	904	775	524	13.4
Albania	-	-	1	4	178	202	5.2
Egypt	154	101	580	954	949	155	3.9
Turkey	62	92	214	355	49	105	2.7
Croatia	263	665	26	4	14	90	2.3
Israel	2	25	50	119	50	48	1.2
Macedonia	22	1	10	25	6	23	0.6
Switzerland	9	111	38	18	29	13	0.3
Serbia	-	-	-	-	23	12	0.3
China	-	-	1	2	7	2	0.1
Other	280	298	17	63	35	2	0.1
TOTAL	**2 586**	**1 977**	**3 055**	**5 264**	**4 530**	**3 922**	**100.0**

Table 8: Intra-community trade in cabbage in metric tons (EUROSTAT)

Receiving country	2007	2008	2009	2010	2011	2012	Share 2012 (%)
Germany	130 447	153 925	155 079	179 927	169 761	150 184	31.5
United Kingdom	75 641	85 108	75 311	77 347	76 163	78 624	16.5
Netherlands	35 245	33 685	31 626	41 023	72 506	54 730	11.5
France	26 414	26 806	33 745	34 844	40 277	51 363	10.8
Belgium	21 199	22 588	27 064	22 409	23 622	25 954	5.4
Italy	10 345	6 928	19 474	11 438	15 614	22 439	4.7
Austria	20 893	19 618	23 804	22 110	18 425	16 780	3.5
Denmark	12 768	15 497	16 852	16 643	15 068	14 103	3.0
Sweden	4 566	4 795	4 422	5 165	7 850	10 064	2.1
Spain	9 727	12 108	10 391	10 683	6 245	8 229	1.7
Poland	5 628	9 745	8 665	8 498	9 996	7 489	1.6
Lithuania	1 136	1 733	2 299	3 363	5 662	6 363	1.3
Czech Republic	3 110	4 061	5 060	4 266	4 921	5 064	1.1
Hungary	2 426	4 588	5 462	4 939	5 927	4 458	0.9
Finland	3 224	3 047	3 290	2 687	3 368	4 104	0.9
Ireland	4 939	2 758	3 187	3 280	2 880	3 609	0.8
Slovenia	3 346	3 270	3 655	3 914	3 668	3 559	0.7
Slovakia	1 037	2 105	1 994	2 052	2 207	2 366	0.5
Portugal	1 493	1 640	1 947	2 231	2 498	2 343	0.5
Romania	1 032	1 423	687	1 238	1 477	1 976	0.4
Luxembourg	1 048	819	457	1 076	1 162	1 081	0.2
Greece	618	1 227	1 600	943	642	523	0.1
Latvia	511	564	332	217	321	459	0.1
Bulgaria	143	167	193	296	185	433	0.1
Cyprus	21	88	39	78	82	338	0.1
Estonia	161	156	137	249	319	202	0.0
Malta	12	46	77	66	75	77	0.0
TOTAL	**377 127**	**418 492**	**436 850**	**460 981**	**490 920**	**476 913**	**100.0**

Table 9: EU production of cabbage in metric tons (FAOSTAT)

Producing Country	2007	2008	2009	2010	2011	Share 2011 (%)
Poland	1 389 200	1 256 470	1 337 350	1 047 000	1 288 740	23.7
Romania	899 245	967 627	1 004 190	983 648	1 027 840	18.9
Germany	777 721	806 078	841 181	787 065	828 517	15.2
Italy	331 204	344 999	338 087	348 762	333 597	6.1
United Kingdom	258 600	279 100	277 500	291 300	279 353	5.1
Netherlands	289 000	308 000	281 500	277 000	249 000	4.6
Greece	168 720	188 200	182 000	188 200	180 100	3.3
Spain	254 530	251 900	200 000	193 600	169 600	3.1
Portugal	150 000	161 000	163 000	170 189	150 734	2.8
France	227 956	225 528	104 216	100 021	113 079	2.1
Lithuania	94 490	117 155	123 314	53 306	112 897	2.1
Austria	98 627	91 882	94 165	91 929	102 318	1.9
Hungary	91 623	103 187	100 170	76 572	101 109	1.9
Belgium	123 500	116 900	102 100	100 801	100 386	1.8
Latvia	51 537	53 435	61 856	60 023	61 204	1.1
Ireland	45 000	45 580	51 587	44 602	59 469	1.1
Czech Republic	42 420	50 700	45 350	35 856	58 386	1.1
Slovakia	77 206	78 602	50 188	46 711	56 825	1.0
Bulgaria	50 000	64 884	39 389	78 939	44 643	0.8
Finland	23 956	22 347	29 999	26 912	28 190	0.5
Denmark	25 000	25 322	27 643	22 710	26 120	0.5
Slovenia	22 200	28 911	30 412	21 195	21 819	0.4
Estonia	19 095	19 751	18 615	16 280	20 648	0.4
Sweden	15 200	16 900	18 000	20 800	17 800	0.3
Extra-EU	8 654	11 836	6 814	7 935	5 688	0.1
Cyprus	4 350	4 397	4 606	4 343	3 778	0.1
Malta	3 311	3 393	3 120	3 334	3 760	0.1
Luxembourg	110	49	59	87	98	0.0
TOTAL	**5 542 455**	**5 644 133**	**5 536 411**	**5 099 120**	**5 445 698**	**100.0**

Table 10: Imports of cabbage from outside the EU in metric tons (EUROSTAT)

Exporting Country	2007	2008	2009	2010	2011	2012	Share 2012 (%)
Tunisia	2 956	4 127	2 957	4 009	3 577	2 561	56.5
Egypt	2 864	3 693	2 350	1 923	1 351	976	21.5
Turkey	645	1 341	335	638	86	331	7.3
United States	1 160	1 750	176	713	-	202	4.4
Croatia	214	170	179	152	125	198	4.4
Morocco	606	548	600	102	327	187	4.1
Serbia	-	-	98	282	99	65	1.4
Macedonia	13	16	19	6	0	7	0.1
Israel	79	24	7	17	55	2	0.0
Others	117	168	94	93	69	1	0.0
TOTAL	**8 654**	**11 836**	**6 814**	**7 935**	**5 688**	**4 529**	**100.0**

Table 11: Intra-community trade in cabbage in metric tons (EUROSTAT)

Receiving country	2007	2008	2009	2010	2011	2012	Share 2012 (%)
Germany	106 617	111 926	107 184	116 475	113 747	86 600	21.6
United Kingdom	98 888	85 418	79 539	75 716	65 861	84 308	21.0
Italy	27 568	29 648	39 258	40 775	49 085	48 173	12.0
Sweden	26 124	29 405	28 920	31 072	30 713	30 140	7.5
France	24 253	24 047	21 374	21 552	21 042	27 313	6.8
Poland	11 319	15 000	15 935	16 543	19 134	18 649	4.6
Finland	15 041	16 172	16 315	17 899	19 016	18 370	4.6
Austria	16 604	16 538	14 869	14 326	12 010	13 589	3.4
Netherlands	17 993	23 183	20 924	19 676	17 423	13 129	3.3
Lithuania	848	3 538	2 862	6 519	8 989	12 894	3.2
Denmark	10 152	12 339	13 094	13 600	13 384	8 144	2.0
Czech Republic	7 896	7 771	8 173	8 319	10 206	7 842	2.0
Ireland	6 348	7 528	6 983	6 431	5 958	6 888	1.7
Spain	7 896	6 773	6 389	7 170	5 437	5 427	1.4
Slovenia	4 576	4 897	5 449	5 004	4 821	5 166	1.3
Belgium	4 269	2 055	3 000	1 375	1 621	2 525	0.6
Hungary	4 834	3 013	1 691	1 816	2 274	2 439	0.6
Romania	966	1 264	1 608	2 385	2 769	2 313	0.6
Slovakia	1 968	3 086	3 659	3 161	3 510	1 952	0.5
Latvia	463	454	604	706	930	1 415	0.4
Estonia	493	668	593	621	766	979	0.2
Greece	2 093	2 318	1 850	2 029	994	959	0.2
Luxembourg	517	635	784	954	940	848	0.2
Portugal	734	303	352	913	303	482	0.1
Bulgaria	42	53	21	55	324	387	0.1
Malta	46	87	154	121	318	322	0.1
Cyprus	183	272	228	251	279	85	0.0
TOTAL	**398 732**	**408 392**	**401 810**	**415 463**	**411 854**	**401 337**	**100.0**

Table 12: EU production of spinach in metric tons (FAOSTAT)

Producing Country	2007	2008	2009	2010	2011	Share 2011 (%)
France	143 487	123 500	78 246	80 101	109 835	19.6
Belgium	100 300	81 000	86 800	93 150	99 750	17.8
Italy	96 418	99 800	89 443	90 608	82 410	14.7
Spain	67 167	59 476	48 400	59 403	70 631	12.6
Germany	61 398	62 472	60 807	49 470	61 257	10.9
Greece	44 064	44 200	50 000	56 100	55 600	9.9
Netherlands	44 000	38 500	32 000	29 500	34 000	6.1
Portugal	16 000	14 853	16 500	17 228	15 259	2.7
Austria	12 148	12 757	10 109	9 018	14 855	2.6
Hungary	2 947	3 310	2 800	1 987	5 382	1.0
Czech Republic	1 791	2 200	1 713	2 200	3 045	0.5
Romania	2 856	1 408	1 412	1 696	2 321	0.4
Slovakia	1 042	2 395	2 028	1 480	2 041	0.4
Extra-EU	1 130	880	1 915	1 931	1 641	0.3
Cyprus	1 495	1 666	322	927	955	0.2
Finland	950	766	752	471	841	0.1
Bulgaria	740	492	433	482	736	0.1
Denmark	-	-	-	440	610	0.1
Malta	267	217	315	282	255	0.0
Slovenia	251	329	364	236	215	0.0
Lithuania	45	56	63	68	81	0.0
Estonia	-	-	-	-	-	0.0
Ireland	-	-	-	-	-	0.0
Latvia	-	-	-	-	-	0.0
Luxembourg	-	-	-	-	-	0.0
Poland	-	-	-	-	-	0.0
Sweden	-	-	-	-	-	0.0
United Kingdom	-	-	-	-	-	0.0
TOTAL	**598 496**	**550 277**	**484 422**	**496 778**	**561 720**	**100.0**

Table 13: Imports of spinach from outside the EU in metric tons (EUROSTAT)

Exporting Country	2007	2008	2009	2010	2011	2012	Share 2012 (%)
Turkey	667	456	1 573	1 624	1 538	673	61.1
United States	190	150	202	214	19	247	22.4
Tunisia	-	-	4	7	27	113	10.3
Egypt	1	1	2	0	7	37	3.4
Thailand	7	23	26	48	16	13	1.2
Norway	-	-	-	10	-	6	0.6
Other	265	250	108	29	35	13	1.2
TOTAL	**1 130**	**880**	**1 915**	**1 931**	**1 641**	**1 102**	**100.0**

Table 14: Intra-community trade in spinach in metric tons (EUROSTAT)

Receiving country	2007	2008	2009	2010	2011	2012	Share 2012 (%)
Belgium	9 578	11 161	14 627	14 011	19 253	25 125	29.3
Netherlands	29 831	30 475	28 087	17 782	13 870	21 289	24.8
Germany	3 453	9 616	5 069	6 551	18 387	17 286	20.1
United Kingdom	10 927	13 377	10 881	10 274	10 363	11 064	12.9
France	1 804	1 701	899	1 522	1 755	2 474	2.9
Sweden	1 283	862	896	1 139	1 366	1 647	1.9
Italy	1 581	1 737	2 086	1 416	1 304	1 129	1.3
Spain	1 069	1 379	832	842	1 453	1 034	1.2
Bulgaria	125	163	14	33	681	1 013	1.2
Ireland	543	322	338	429	605	598	0.7
Poland	123	183	193	326	387	536	0.6
Austria	232	317	324	653	605	506	0.6
Czech Republic	250	267	330	401	442	448	0.5
Slovakia	12	59	48	39	59	350	0.4
Romania	121	157	24	233	114	249	0.3
Portugal	420	222	365	529	609	187	0.2
Lithuania	39	51	42	81	101	172	0.2
Denmark	236	567	445	617	440	158	0.2
Greece	266	397	136	195	183	122	0.1
Luxembourg	66	104	128	100	97	114	0.1
Finland	54	61	67	81	102	111	0.1
Slovenia	94	193	123	154	111	105	0.1
Latvia	24	52	24	30	43	80	0.1
Hungary	15	5	1	56	10	20	0.0
Estonia	9	8	5	7	4	6	0.0
Malta	0	0	1	3	3	2	0.0
Cyprus	0	1	1	0	0	0	0.0
TOTAL	**62 151**	**73 438**	**65 985**	**57 503**	**72 346**	**85 822**	**100.0**

GLOSSARY

Clean water is clean seawater (natural, artificial or purified seawater or brackish water that does not contain microorganisms, harmful substances or toxic marine plankton in quantities capable of directly or indirectly affecting the health quality of food) and fresh water of a similar quality (Regulation (EC) No 852/2004)[49].

Decontamination treatments are mechanical, physical, and chemical treatments, which are applied to eliminate contaminants, including microbial contamination. They can be applied to water, surfaces, equipment and areas.

Disinfectants are agents or systems that kill or eliminate bacteria found on inanimate surfaces or environments. Within this opinion, disinfectant agents or systems are defined as those decontamination agents applied to eliminate microorganisms in wash water.

Fertigation is the application of fertilizers, soil amendments, or other water-soluble products through an irrigation system.

Food of non-animal origin include those derived from plants and comprise a wide range of fruit, vegetables, salads, juices, seeds, nuts, cereals, herbs, spices, fungi and algae, which are commonly consumed in a variety of forms. Categorisation of FoNAO, as considered in the scope of this Opinion, is discussed in Chapter 2.2 of EFSA Panel on Biological Hazards (BIOHAZ) (2013).

Food Safety Criteria are defined in EU legislation for the microbiological acceptability of food products and are criteria defining the acceptability of a product or a batch of foodstuff applicable to products placed on the market (Regulation (EC) No 2073/2005)[50]. If a Food Safety Criterion is not met for a product or batch of foodstuff, then this should not be placed on the market or, if it already has, be considered for recall.

Fresh Produce refers to fresh fruits and vegetables that are likely to be sold to consumers in an unprocessed or minimally processed (i.e. raw) form and are generally considered as perishable. Fresh produce may be intact, such as strawberries, whole carrots, radishes, and fresh market tomatoes, or cut during harvesting, such as celery, broccoli, and cauliflower[51]. In the scope of this opinion fresh produce also applies to fresh-cut produce, such as pre-cut, packaged, ready-to-eat salad mixes.

Good Agricultural Practices (GAP) apply available knowledge to address environmental, economic and social sustainability for on-farm production and post-production processes resulting in safe and healthy food and non-food agricultural products (FAO, 2003).

Good Hygiene Practices (GHP) relate to general, basic conditions for hygienic production of a foodstuff, including requirements for hygienic design, construction and operation of the plant, hygienic construction and use of equipment, scheduled maintenance and cleaning, and personnel training and hygiene. A developed and implemented GHP programme is a pre-requisite for HACCP system (EFSA, 2005).

Good Manufacturing Practices (GMP) cover the principles needed to design plant layout, equipment and procedures for the production of safe food. This includes hygienic operation and cleaning and disinfection procedures. The codes and requirements may be formally specified by e.g. Codex Alimentarius Committee on Food Hygiene (EFSA, 2005).

[49] Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. OJ L 139, 30.4.2004, p.1-54

[50] Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. OJ L 338, 22.12.2005, p.1-26

[51] FDA Guidance for Industry: guide to minimize microbial food safety hazards for fresh fruits and vegetables. 1998. http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ProducePlantProducts/ucm064574.htm

Harvest is the process of collecting mature crops from the fields and immediate handling.

Hygiene Criteria are criteria indicating the acceptable functioning at pre-harvest, harvest and on farm post-harvest production prior to processing and are proposed to verify and validate Good Agricultural Practices (GAP) and Good Hygiene Practices (GHP).

Leafy greens are leaves, stems and shoots from various leafy plants which are eaten as vegetables, and for the purposes of this opinion, only those eaten raw will be considered.

Minimal processing is any action applied to the initial product (e.g. cleaning, coring, peeling, chopping, slicing or dicing and washing) and which is not included below in the definition of processing (e.g. heating, smoking, curing, maturing, drying, marinating, extraction, extrusion or a combination of those processes). Minimal processing may occur at harvest as well as on farm post-harvest and at processing.

Potable water is water which meets the requirements laid down in Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption (mainly microbiological and chemical criteria) (Regulation (EC) No 852/2004)[52].

Post-harvest is the stage of crop production after harvest and includes on-farm cooling, cleaning, sorting and packing.

Pre-harvest incorporates all activities on the farm that occur before crop products are harvested.

Process Hygiene Criteria are criteria indicating the acceptable functioning of the production process. Such criteria are not applicable to products placed on the market. They set an indicative contamination value above which corrective actions are required in order to maintain the hygiene of the process in compliance with food law (Regulation (EC) No 2073/2005)[53].

Processing are any actions that substantially alter the initial product, including heating, smoking, curing, maturing, drying, marinating, extraction, extrusion or a combination of those processes (Regulation (EC) No 852/2004)[54].

Sanitizers are chemical agents that reduce microorganisms on food contact surfaces by at least 99.999 %. Within this opinion sanitizers are defined as those decontamination agents applied to reduce the level of microorganisms on leafy greens.

Silique is an elongated fruit composed of two carpels separated by a seed-bearing partition.

[52] Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. OJ L 139, 30.4.2004, p.1-54.
[53] Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. OJ L 338, 22.12.2005, p.1-26.
[54] Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. OJ L 139, 30.4.2004, p.1-54.